THE UNIVERSITY OF ALBERTA

STUDIES ON (1) NITROGEN METABOLISM IN THE HINDGUT OF THE GROWING PIG AND (2) COMPOSITION AND UTILIZATION OF HIGH LYSINE BARLEYS BY GROWING RATS AND PIGS

by

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A THESIS

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GENERAL ABSTRACT

A. NITROGEN METABOLISM IN THE HINDGUT OF THE GROWING PIG

Four experiments were conducted with growing pigs (30 to 50 kg initial liveweight), each fitted with a single T-shaped cannula at the end of the small intestine, approximately 5 cm from the ileocecal valve, to evaluate the significance in practical nutrition of the role of the hindgut in nitrogen (N) metabolism. In the first study (Chap. II), pigs were fed meat-and-bone meal- or soybean meal-supplemented cornstarch (CS)-based diets; in the second study (Chap. III), barley or wheat–based diets; in the third study (Chap. IV), a soyprotein (SP)-supplemented CS-based diet. The diets provided adequate or slightly below adequate crude protein. Energy substrates (purified carbohydrates — CS or pectin — or a "natural-type" carbohydrate — wheat bran, WB), protein substrates (SP or WB), and a CS plus SP combination were infused through the cannulas. Results indicated that N metabolism in the hindgut, as a consequence of microbial activity, was affected by infusions of both carbohydrate and protein. A high energy/N ratio elicited an intensification of microbial activity, resulting in quantitative changes in the route of N excretion. Greater output of fecal N was accompanied by decreases in total urinary N. The hindgut was shown to possesses a high capacity for N digestion; however, this digestion did not contribute to the N status of the pigs, as was demonstrated by no increase in N retention and apparent biological value. The N end products of hindgut

protein digestion were absorbed into the blood and excreted mainly as urea in the urine. Wide variations in the apparent digestibilities of N (crude protein) and constituent amino acids (AA), as determined by the fecal analysis method, emphasized the importance of considering digestibilities of AA, and not crude protein in diet formulation. Furthermore, because of microbial activity in the hindgut, the fecal analysis method underestimated or overestimated AA availabilities. The problems associated with this method for obtaining reliable estimates of AA availabilities in dietary protein (of lower ileal digestibility) may be overcome by measuring digestibilities of AA in ileal digesta.

B. COMPOSITION AND UTILIZATION OF HIGH LYSINE BARLEYS

Chemical analyses and biological evaluation, using rats and /or pigs, were conducted on samples of six Alberta-bred barley lines, plus one established high protein high lysine barley (Hiproly), and one high lysine mutant (Risø 1508), all grown in Alberta under similar soil and environmental conditions. Results showed that Line 1 could be classified as a "hiproly" barley; Line 2, a "high protein" barley; Lines 4 and 6, "high lysine" barleys; and Lines 3 and 5, "normal" barleys. Chemical score data showed that Line 1 and Risø 1508 could meet the total AA requirements for 60 to 100 kg pigs in practical diets. Lines 2, 4 and 6, and Hiproly as compared to Galt (control barley), would require reduced protein supplementation. Feeding trials with rats (Chap. V),

and both rats and pigs (Chap. VI) showed that the digestibility of lysine was similar to or greater than that of the corresponding Galt barley. The superior nutritional quality of Hiproly, Risø 1508 and Line 6 relative to that of Galt, as measured both by relative protein value in rats, and N retention and apparent biological value in pigs, could be related directly to the amount of available lysine.

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DEDICATION

To my son, Anil Jainarayan,

And daughters, Renuka Devi and Nina Roshini.

Let the light shine!

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I. GENERAL INTRODUCTION

SCOPE OF THIS THESIS

Two aspects of nutritional research were studied in experiments with pigs and rats. The first and major study was designed to elucidate the role of the hindgut of the growing pig in the metabolism of nitrogen (N). The second study involved chemical and nutritional evaluation of high lysine barleys and locally bred barley lines, all grown in Alberta. The material in this thesis is therefore presented in two sections.

A. NITROGEN METABOLISM IN THE HINDGUT OF THE GROWING PIG

Digestion involves the breakdown of diverse, complex molecules of dietary components into simple molecules suitable for absorption. Digestive processes are facilitated both by the action of endogenously produced enzymes, and enzymes produced by microorganisms which exist in symbiotic relationship with the host animal. In the case of the pig, microbial activity is confined mainly to that region of the intestinal tract posterior the end of the small intestine, i.e., the hindgut.

The hindgut (which includes the cecum, colon and rectum) is the last region of the intestinal tract through which digesta from the small intestine must pass before being voided as feces. In the pig, the hindgut accounts for about 38% of the volume of the intestinal tract (Bayley 1978), and at any given time contains 30 to 60% of the total

weight of the digesta (Friend et al. 1963, Ledinek 1970). In addition, the mean transit time is 30 to 36 hours in the hindgut, as compared to only 3 to 5 in the stomach or the small intestine (Keys and Debarthe 1974). Under these conditions in the hindgut, absorption of water (Low et al. 1978), minerals (Partridge 1975) and vitamins (Hotzel and Barnes 1966) are facilitated. In addition, there is microbial proliferation.

A great diversity of microorganisms inhabit the hindgut of the pig. The number of microbes/g of fresh digesta may reach 10^9 for aerobes, and 10^{11} for obligate anaerobes (Ledinek 1970, Koch et al. 1972). In preweaned pigs, microbes frequently isolated include lactobacilli and yeasts (Ledinek 1970, Koch et al. 1972). In older animals, gram negative anaerobes become established and predominate (Schaedler 1973). Different feeding regimes may have an influence on microbial growth, but little effect on the composition of the microfloral population (Drasar et al. 1973).

The microbes degrade undigested nutrients entering the hindgut, including protein and carbohydrate residues (Misir and Sauer 1980, 1981a,b), and endogenous N substrates such as amino acids (Holmes et al. 1974) and urea (Deguchi et al. 1979, Bergner 1981, Mosenthin 1981). An increase in microbial activity was demonstrated by the excretion of greater amounts of fecal N (Mason and Palmer 1973, Mason et al. 1976, Mendez-Pereira et al. 1977), fecal bacterial

diaminopimelic acid (Mason and Palmer 1973, Mason et al. 1976) and bacterial ribonucleic acid (Mason et al. 1976, Gargallo and Zimmerman 1981).

In practical production, pigs are fed diets consisting of cereal grains plus protein supplements (e.g., canola meal, meat-and-bone meal and soybean meal) which supply amino acids deficient in the grains. Feed costs may account for 50 to 70%, or even more, of total cost of producing a market pig. It is therefore incumbent for the animal nutritionist to consider not only the nutrient composition of the diet but, more importantly, the extent to which these nutrients are available to the animal. Recommendations for the inclusion of protein and amino acids in animal diets are often based on the fecal analysis method (Kuiken and Lyman 1948) which ignores the effects of hindgut fermentation. A clear understanding of the role of the hindgut of the pig in N metabolism would allow nutritionists to determine more accurately, and therefore recommend more precisely, dietary allowances for amino acids to meet adequately the animal's requirements for optimal growth.

B. COMPOSITION AND UTILIZATION OF HIGH LYSINE BARLEYS BY GROWING RATS AND PIGS

Barley ranks fourth in world production of cereal grains, preceded by corn, rice and wheat (FAO 1979). Wheat and rice are utilized mainly in human diets, whereas barley and much of the corn are currently fed to domestic livestock

species.

The quality of cereal proteins is generally inferior to animal proteins because of lower levels of indispensable amino acids, resulting in relatively lower biological values. In barley, lysine is the first limiting amino acid (Sauer 1976, Eggum 1977). An increase in the lysine content of the grain is expected to improve the nutritional value and, therefore, reduce the quantity of added protein ingredients required to formulate balanced livestock diets.

In most varieties of barleys ("normal" barleys), the levels of protein and lysine (grain dry matter basis) are positively correlated; however, the amount of protein in the grain and the lysine content of the grain protein are negatively correlated. "High lysine" barleys have higher levels of lysine than normal barleys with the same protein content (Doll et al. 1974).

Concerted efforts by plant breeders in various countries to improve the quality of cereal proteins have resulted in the isolation of several high lysine mutants in corn (Mertz et al. 1964, Nelson et al. 1965), sorghum (Singh and Axtell 1973) and barley (Bansal 1970, Munck et al. 1971, Ingversen et al. 1973). Barley mutants, such as Hiproly (Munck et al. 1971), Risø 1508 (Ingversen et al. 1973) and Notch-1 and Notch-2 (Balaravi et al. 1976) have higher content of lysine and reduced levels of glutamic acid and proline. This altered amino acid profile was achieved by an increase in the levels of lysine-rich albumin and globulin

protein fractions at the expense of lysine-poor prolamines (Doll and Koie 1973, Ingversen et al. 1973, Balaravi et al. 1976). Feeding experiments with rats (Munck 1972, Doll et al. 1974, Balaravi et al. 1976) and pigs (Thomke and Widstromer 1975, Newman et al. 1978) attest to the nutritional superiority of high lysine barleys as compared to normal cultivars. However, most if not all, high lysine barleys have certain undesirable agronomic characteristics, e.g., reduced grain size and therefore low grain yield (Doll and Koie 1973, Ingversen et al. 1973, Bansal et al. 1977). As a consequence, researchers involved in barley breeding programs are currently attempting to combine the high lysine trait with desirable agronomic traits, including high yield. In Alberta, this program is aimed at producing high yielding high lysine barleys specifically adapted to local conditions. It is anticipated that the development of such barleys would make a significant contribution towards more efficient livestock production by improving feed conversion efficiency, thereby reducing total feed costs, and resulting in more profit to producers.

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II. EFFECT OF STARCH INFUSION AT THE TERMINAL ILEUM ON NITROGEN BALANCE AND APPARENT DIGESTIBILITIES OF NITROGEN AND AMINO ACIDS IN GROWING PIGS [1]

A. ABSTRACT

Growing pigs (33 to 38 kg initial liveweight) were each surgically fitted with a single T-shaped cannula at the end of the small intestine, and fed a cornstarch-based diet made isonitrogenous with meat-and-bone meal (MBM) or soybean meal (SBM). Starch (200 g/d) or water (400 g/d) was infused through the cannula and effects on nitrogen (N) balance and apparent digestibilities (AD) of amino acids (AA) were studied. The infusion of starch, as contrasted to water, increased ($P<0.01$) excretion of fecal N and correspondingly decreased ($P<0.05$) excretion of total urinary N, including urinary urea N. There was no effect ($P>0.05$) on the amount of N retained. The increased excretion of fecal AA following starch infusion resulted in lowered ($P<0.05$) AD (percentage units) of indispensable AA: threonine (8.4), methionine (6.7), valine (6.6) and lysine (5.0); and dispensable AA: tyrosine (6.9) and aspartic acid (5.7). In general, the same pattern of AD decreases was obtained for both protein

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sources; however, the magnitude of these decreases tended to be greater for MBM than for SBM. There were large differences (percentage units) between AD of N (crude protein) and those of individual AA (arginine +11.2, and isoleucine -5.2, in MBM; arginine +7.6, and methionine -7.6, in SBM). Results showed that the route of N excretion and, consequently, the AD of AA, as determined by the fecal analysis method, were affected by the amount of starch (energy substrate) entering the hindgut. It is concluded that the formulation of pig diets could be done more accurately by using AD of individual AA, and not N; however, because of the dependence of AD values on the amount of starch in the hindgut, AD values (fecal analysis method) may not be reliable indices of AA availabilities for pigs.

B. INTRODUCTION

The digestibility of the AA in a given protein supplement in diets for monogastric animals is often determined by the fecal analysis method (Kuiken and Lyman 1948) which considers the amounts of specific AA consumed and voided in the feces, but does not take into account the modifying action of the microflora in the hindgut. Several workers reported considerable disappearance of N in the hindgut of chicks (Nesheim and Carpenter 1967, Salter and Fulford 1974), rats (Mason and Palmer 1973, Eggum et al. 1979), and pigs (Zebrowska 1973, 1975, Hodgdon 1977, Sauer et al. 1977a,b, 1979, 1980, Mason 1979). Furthermore, in the

case of the pig, other workers indicated a net increase in the amounts of AA in the hindgut, e.g., lysine and methionine (Sauer et al. 1980), lysine and isoleucine (Sauer et al. 1977b) and arginine, methionine, cysteine and tyrosine (Holmes et al. 1974). Consequently, AA digestibilities, as determined by the fecal analysis method, may not reflect the amounts actually available for absorption and subsequent protein synthesis.

Microbial activity in the hindgut may be limited by the presence of energy substrates (Mason et al. 1976). The capacity of the hindgut for starch digestion (g/d) was estimated at 153 for sheep (Mason et al. 1977), or 150 for pigs (Gargallo and Zimmerman 1981). When the less digestible potato starch as compared to cornstarch was fed to pigs (Cunningham et al. 1963, Mason et al. 1976) more undigested starch entered the hindgut and thereby increased the level of bacterial activity (Mason and Palmer 1973, Mason et al. 1976).

The objective of this study was to determine the extent to which the infusion of excess starch into the hindgut would affect N balance and apparent fecal digestibilities of N and AA when MBM and SBM were fed to growing pigs as sole protein sources.

C. MATERIALS AND METHODS

Six Yorkshire x Lacombe barrows, ranging in initial body weight from 33 to 38 kg, were each surgically fitted with a single T-shaped cannula at the end of the small intestine, approximately 5 cm from the ileocecal junction. Each cannula (internal and external diameters of 12 and 18 mm, respectively) was made of polyvinylchloride plastisol, according to procedures outlined by Sauer (1976). Following surgery the pigs were allowed a 21-d recuperation period during which they were fed ad libitum a ground barley-SBM grower diet (1 mm particle size) formulated to 14% crude protein. The pigs were confined to stainless steel metabolic cages that permitted separate collection of feces and urine. The barn was provided with continuous lighting and maintained at temperature and relative humidity of 20 to 22°C and 70 to 75%, respectively.

Each of the experimental periods (n=2) consisted of a 5-d adaptation period followed immediately by a 5-d collection period. At the commencement of the experiments, pigs (n=3) were randomly selected and fed cornstarch-based diets (n=2) made isonitrogenous with MBM and SBM (Table II.1). Vitamins and minerals were added to meet or exceed NRC (1979) specifications. Each pig was given a slurry of 800 g diet and 2 litres of water daily at 0800 h and 1600 h throughout the experiment. Additional water was provided ad libitum. Within 1 h of feeding, infusions (n=2) of water or a slurry of soluble starch (Fisher Scientific Co., Fair

Table II.1. Composition and partial chemical analyses of the basal diets.

Diets:	Meat-and-bone meal	Soybean meal
Ingredients (%, as fed)		
Corn starch	63.68	62.78
Meat-and-bone meal (49.0% CP)	28.60	-
Soybean meal (48.0% CP)	-	30.50
Tallow	4.00	4.00
Calcium carbonate (38% Ca)	2.20	-
Calcium phosphate (17% Ca; 21% P)	0.80	2.00
Trace mineralized salt[1]	0.50	0.50
Trace mineral premix[2]	0.15	0.15
Vitamin premix[3]	0.015	0.015
Choline chloride	0.055	0.055
Chemical analyses (as fed basis)[4]		
Dry matter, %	92.45±0.03	91.90±0.25
Nitrogen, %	2.22±0.02	2.13±0.01
Gross energy, MJ/kg	16.38±0.04	17.06±0.03

[1]Supplied by Windsor Salt Co., Toronto, Canada. The composition (percentage) was: NaCl, 96.5; ZnO, 0.40; $FeCO_3$, 0.16; MnO, 0.12; CuO, 0.033; $Ca(IO_3)_2$, 0.007; CoO, 0.004.

[2]Contributed the following nutrients in milligrams per kilogram of diet: Zn, 100; Cu, 10; Mn, 20; Fe, 150; Se, 0.10.

[3]Contributed the following vitamins per kilogram of diet: vitamin A, 1,300 IU; vitamin D_3, 150 IU; vitamin E, 11 IU; menadione, 2 mg; biotin, 0.1 mg; folic acid, 0.6 mg; niacin, 12 mg; pantothenic acid, 11 mg; pyridoxine, 1.1 mg; riboflavin, 2.2 mg; thiamine, 1.1 mg, and vitamin B_{12}, 11 micrograms.

[4]Mean ± standard error.

Lawn, NJ, USA) was administered into the cannula of each pig, using a 50 ml catheter-tip syringe (Table II.2, footnotes 1 and 2). The infusion pattern followed in period 1 was reversed in period 2 (Table II.2). The average initial and final weights of the pigs during the experiment were 46 and 54 kg, respectively.

The feces produced were carefully separated from hair and spilled feed. Total feces output was collected once daily and placed into separate polyethylene bags. The urine was collected in plastic pails containing 200 ml of 5% hydrochloric acid; a daily check was made to ensure the urine pH was maintained below 5.0. After measuring the daily output for each pig, a 1% subsample was placed into a stoppered polyethylene bottle. All feces and urine samples were frozen and stored at -20°C until required for analysis. Samples of diets (about 5g) were taken when the daily allowances were weighed into individual paper bags at the start of each experimental period.

Chemical Analyses

Air dry samples of MBM, SBM, diets and composite freeze-dried feces were ground to pass through a 0.8 mm screen prior to analyses. The diets were analyzed for dry matter, Kjeldahl N and gross energy; and the feces for dry matter and Kjeldahl N. The starch used in the infusions was analyzed for gross energy; filtered urine samples for Kjeldahl N (AOAC 1970). The urea content of urine samples, pooled among days for each pig during both periods, was

Table II.2. Experimental design and infusion pattern.

Diet	Pig No.	Material Infused	
		Period 1	Period 2
Meat-and-bone meal	1	S[1]	H_2O
	2	H_2O[2]	S
	3	S	H_2O
Soybean meal	4	H_2O	S
	5	S	H_2O
	6	H_2O	S

[1]S = starch (100 g/200 ml slurry, temperature 37°C approximately; gross energy infused was 3.14 MJ/d).

[2]H_2O = water (200 ml, temperature 37°C approximately).

determined with an autoanalyzer (Anonymous 1974). In addition, duplicate feed samples and single fecal samples (pooled among days) from all the pigs during both periods were analyzed for AA content, as described by Sarwar and Bowland 1975.

Calculations and Statistical Analysis

For each pig, feed intake was determined by subtracting the total feed spillage from the amount supplied. Apparent digestibilities (AD) of dry matter, crude protein and individual AA were calculated as the differences between the respective amounts (g) consumed and voided in the feces, and expressed as percentages of the total nutrient intakes (Kuiken and Lyman 1948). The retained N was taken as that proportion of the intake N not excreted in feces and urine.

The data for each response criterion were subjected to least squares analyses of variance (Mehlenbacher 1978). Differences among treatment means were determined according to the Student-Newman-Keuls' multiple range test (Snedecor and Cochran 1967).

D. RESULTS

Analyses of the diets showed a greater recovery of N from AA, and similar or higher levels of most indispensable AA in the SBM diet rather than the MBM diet (Table II.3). Mean intakes of N during the 5-d experimental period were 171.4 and 164.2 g by the pigs fed MBM and SBM diets, respectively (Table II.4). This difference was less than 5%,

Table II.3. Partial amino acid composition of the diets.

Diets:	Meat-and-bone meal	Soybean meal
Amino acids, % dry matter		
Indispensable		
Arginine	1.06	1.08
Histidine	0.29	0.42
Isoleucine	0.44	0.70
Leucine	0.93	1.19
Lysine	0.82	0.99
Methionine	0.24	0.23
Phenylalanine	0.51	0.76
Threonine	0.50	0.62
Valine	0.62	0.75
Dispensable		
Alanine	1.18	0.64
Aspartic acid	1.11	1.73
Cysteine	0.08	0.24
Glutamic acid	2.01	2.97
Glycine	2.03	0.62
Proline	1.38	0.83
Serine	0.58	0.74
Tyrosine	0.23	0.36
Amino acid N recovery, %[1]	87.38	95.00

[1]Average of two analyses.

Table II.4. Effect of protein source and infusion at the terminal ileum on nitrogen balance and urinary urea nitrogen in pigs.

Factor	Intake N	Fecal N	Urinary N	Retained N	Urinary Urea N
	—g/5d —	— % of Intake N —			
Protein source (P)					
MBM[1]	171.4	28.5^{A}[3]	47.7^{A}	23.8^{B}	15.6^{A}
SBM[2]	164.2	16.4^{B}	33.3^{B}	50.3^{A}	10.8^{B}
SEM[4]	-	0.87	1.52	1.10	0.24
Infusion (I)					
H_2O	169.4	20.7^{B}	42.4^{a}[3]	36.9	14.2^{a}
Starch	166.2	24.2^{A}	38.5^{b}	37.3	12.3^{b}
SEM	-	0.26	0.73	0.86	0.24
P X I					
MBM x H_2O	174.6	26.1	51.3	22.5	17.4[5]
MBM x starch	168.2	30.9	44.1	25.0	13.8
SBM x H_2O	164.2	15.3	33.5	51.2	10.9
SBM x starch	164.3	17.5	33.0	49.5	10.7
SEM	-	0.38	1.05	1.23	0.34

[1]MBM = meat-and-bone meal

[2]SBM = soybean meal.

[3]For each factor, means within a given column not followed by the same superscript are significantly different: A,B at $P<0.01$; a,b at $P<0.05$.

[4]Standard error of the mean.

[5]Interaction is significant at $P<0.05$.

and therefore was not expected to affect any of the parameters measured.

When the pigs were fed the MBM, as compared to the SBM diet, losses of both fecal N and total urinary N were greater, and the amount of N retained lower ($P<0.01$). In addition, the level of urea N excreted in the urine was higher for MBM ($P<0.01$, Table II.4). Lower AD ($P<0.01$) were obtained for dry matter, N and the indispensable AA, except methionine (Table II.5), and most of the dispensable AA, including aspartic acid, glutamic acid, proline, serine and tyrosine ($P<0.01$), and cysteine ($P<0.05$); however, the AD of glycine was higher ($P<0.05$, Table II.6).

The infusion of starch, as contrasted to water, was accompanied by an increase ($P<0.01$) in fecal N and a corresponding decrease ($P<0.05$) in total urinary N output. In addition, starch infusion decreased ($P<0.05$) the amount of urea N excreted in the urine but had no effect ($P>0.05$) on the amount of N retained (Table II.4). Furthermore, starch infusion depressed the AD of N ($P<0.01$) and all indispensable AA ($P<0.05$) except histidine (Table II.5), and most dispensable AA, including glutamic acid and serine ($P<0.01$) as well as alanine, aspartic acid, glycine, proline and tyrosine ($P<0.05$, Table II.6). Among the indispensable AA, the largest decreases (percentage units) were found for threonine (8.4), methionine (6.7), valine (6.6) and lysine (5.0); among the dispensable AA, the largest decreases were obtained for tyrosine (6.9) and aspartic acid (5.7).

Table II.5. Effect of protein source and infusion at the terminal ileum on the apparent digestibilities of dry matter, nitrogen and indispensable amino acids (%).

Factor	DM[1]	N	Arg	His	Ile	Leu	Lys	Met	Phe	Thr	Val
Protein source (P)											
MBM	79.7^{B}[2]	71.5^{B}	82.7^{B}	79.5^{B}	66.3^{B}	73.6^{B}	70.9^{B}	72.2	73.2^{B}	67.2^{B}	69.7^{B}
SBM	93.4^{A}	83.6^{A}	91.2^{A}	89.8^{A}	83.8^{A}	85.6^{A}	83.8^{A}	76.0	86.6^{A}	84.2^{A}	80.1^{A}
SEM[3]	0.11	0.64	0.56	0.55	1.61	1.25	1.09	1.68	1.27	1.92	1.29
Infusion (I)											
H_2O	86.0	79.3^{A}	87.8^{a}[2]	86.6	77.2^{a}	81.3^{a}	79.9^{a}	77.5^{a}	82.0^{a}	79.9^{a}	78.2^{a}
Starch	87.1	75.8^{B}	86.1^{b}	82.6	72.9^{b}	77.9^{b}	74.9^{b}	70.8^{b}	77.9^{b}	71.5^{b}	71.6^{b}
SEM	0.27	0.26	0.39	1.63	0.64	0.52	0.81	1.13	0.58	1.27	0.88
P x I											
MBM x H_2O	78.7	73.9	83.4	80.8	69.2	75.9	73.5	76.0	76.0	70.5	74.1
MBM x starch	80.8	69.1	82.0	78.1	63.3	71.3	68.4	68.5	70.4	64.0	65.3
SBM x H_2O	93.4	84.7	92.3	92.5	85.2	86.7	86.3	79.0	87.9	89.3	82.3
SBM x starch	93.5	82.5	90.2	87.1	82.4	84.4	81.4	73.0	85.3	79.1	78.0
SEM	0.38	0.38	0.56	2.34	0.93	0.75	1.16	1.63	0.83	1.82	1.27

[1]Dry matter. The infused starch was considered as dry matter intake for calculation of dry matter digestibilities.

[2]For each factor, means in a given column not followed by the same superscript are significantly different: A,B at $P<0.01$; a,b at $P<0.05$.

[3]Standard error of the mean.

Table II.6. Effect of protein source and infusion at the terminal ileum on apparent digestibilities of dispensable amino acids (%).

Factor	Ala	Asp	Cys	Glu	Gly	Pro	Ser	Tyr
Protein source (P)								
MBM	78.8	69.7^{B}[1]	50.4^{b}[1]	76.7^{B}	85.4^{a}	85.6^{B}	73.6^{B}	56.3^{B}
SBM	77.3	87.0^{A}	93.2^{a}	90.8^{A}	81.8^{b}	91.8^{A}	87.8^{A}	78.4^{A}
SEM[2]	0.88	1.26	7.25	0.91	0.57	0.89	1.16	2.07
Infusion (I)								
H_2O	80.0^{a}	81.2^{a}	76.2	85.4^{A}	84.6^{a}	90.1^{a}	82.5^{A}	70.8^{a}
Starch	76.2^{b}	75.5^{b}	67.4	82.1^{B}	82.7^{b}	87.2^{b}	78.8^{B}	63.9^{b}
SEM	0.67	0.53	6.85	0.37	0.36	0.63	0.34	1.19
P x I								
MBM x H_2O	81.1	73.9[3]	58.1	79.0	86.3	85.7	76.4[3]	61.5
MBM x starch	76.5	65.4	42.7	74.4	84.6	85.4	70.7	51.0
SBM x H_2O	78.8	88.4	94.4	91.8	82.8	94.6	88.6	80.1
SBM x starch	75.8	85.6	92.1	89.8	80.8	89.0	87.0	76.8
SEM	0.96	0.76	9.84	0.53	0.52	0.90	0.49	1.72

[1]For each factor, means within a given column not followed by the same superscript are significantly different: A,B at $P<0.01$; a,b at $P<0.05$.

[2]Standard error of the mean.

[3]Interactions are significant at $P<0.05$.

There was no interaction of protein source x infusion for fecal N, urinary N, retained N (Table II.4) and the AD of dry matter, N, indispensable AA (Table II.5) and most dispensable AA (Table II.6), suggesting a similar pattern of response to starch infusion when the pigs were fed either protein source. However, there were interactions ($P<0.05$) for urinary urea N (Table II.4) and AD of two of the dispensable AA: aspartic acid and serine (Table II.6). Starch infusion depressed each measure to a greater extent when MBM rather than SBM was included in the diet; corresponding decreases (percentage units) were: urinary urea N (3.6 vs 0.2), aspartic acid (8.5 vs 2.8) and serine (5.7 vs 1.6).

The daily output of fecal dry matter was greater when the pigs were infused with water and fed MBM as compared to SBM (i.e., 247.6 vs 78.1 g, respectively, Table II.7). The infusion of starch elicited a greater increase in fecal dry matter output by the MBM-fed pigs (10.5%) than by the SBM-fed pigs (5.6%). There were differences ($P<0.05$) between the feces from pigs fed the MBM diet and feces from pigs fed the SBM diets in content of isoleucine, leucine, methionine, phenylalanine, glutamic acid, glycine, proline and tyrosine, indicating quantitative rather than qualitative effects of starch infusion on AA metabolism in the hindgut (Table II.7). The effect of starch infusion on the apparent increase (appearance) of N and AA (g/d) in the hindgut is presented in Table II.8. The value for N and those for the

Table II.7. Effect of infusion at the terminal ileum on feces dry matter, N and amino acid composition of pigs fed two protein sources.

Diets: Infusion:	Meat-and-bone meal H_2O	Meat-and-bone meal Starch	Soybean meal H_2O	Soybean meal Starch	SEM[1]
Dry matter, g/pig/d	247.6	273.7	78.1	82.5	-
Nitrogen, %[2]	2.94	3.29	5.59	5.68	-
Amino acids, g/16 g N					
Indispensable					
Arginine	4.40	4.20	3.88	4.03	0.19
Histidine	1.35	1.44	1.58	1.99	0.18
Isoleucine	3.42[b3]	3.56[b]	4.81[a]	4.72[a]	0.23
Leucine	5.57[b]	5.79[b]	7.26[a]	7.10[a]	0.29
Lysine	5.42	5.76	6.43	6.95	0.37
Methionine	1.42[b]	1.62[b]	2.17[a]	2.37[a]	0.14
Phenylalanine	3.04[b]	3.29[b]	4.31[a]	4.28[a]	0.23
Threonine	3.59[a]	3.99[ab]	4.82[a]	4.84[a]	0.22
Valine	4.32[b]	5.10[ab]	6.19[a]	6.28[a]	0.34
Dispensable					
Alanine	5.60	6.00	6.22	6.10	0.21
Aspartic acid	7.30[b]	8.43[ab]	9.33[a]	9.59[a]	0.38
Cysteine	1.22[a]	1.04[ab]	0.92[ab]	0.74[b]	0.05
Glutamic acid	10.58[b]	11.19[b]	11.34[a]	11.64[a]	0.41
Glycine	7.05[a]	6.82[a]	4.84[b]	4.63[b]	0.25
Proline	4.80[a]	4.54[a]	3.42[b]	3.37[b]	0.22
Serine	3.44	3.73	3.93	3.72	0.19
Tyrosine	2.18[b]	2.43[b]	3.30[a]	3.16[a]	0.20
Amino acid N recovered[2]	74.09	78.41	84.29	85.14	-

[1]Standard error of the mean.

[2]Average of three analyses.

[3]For a given amino acid, means not followed by a common superscript are significantly different at $P<0.05$.

Table II.8. Effect of starch infusion at the terminal ileum on the apparent increase in fecal nitrogen and amino acid content of pigs fed two protein sources.

Diets:	Meat-and-bone meal	Soybean meal
Nitrogen, g/d[1]	1.71	0.32
Amino acids, g/d[1]		
Indispensable		
Arginine	0.35	0.12
Histidine	0.20	0.16
Isoleucine	0.44	0.08
Leucine	0.74	0.10
Lysine	0.76	0.28
Methionine	0.26	0.10
Phenylalanine	0.47	0.07
Threonine	0.61	0.10
Valine	0.92	0.15
Dispensable		
Alanine	0.82	0.09
Aspartic acid	1.41	0.27
Cysteine	0.06	0.02
Glutamic acid	1.49	0.31
Glycine	0.61	0.03
Proline	0.37	0.05
Serine	0.55	0.10
Tyrosine	0.38	0.02

[1]Average of 3 pigs per infusion (H_2O versus starch) for each diet.

AA were greater when pigs were fed MBM rather than SBM. Among the indispensable AA, the values ranged from 0.20 (histidine) to 0.92 (valine) for MBM-fed pigs, and 0.07 (phenylalanine) to 0.28 (lysine) for SBM; among the dispensable AA, 0.06 or 0.02 (cysteine) to 1.49 or 0.31 (glutamic acid) for pigs fed MBM or SBM, respectively.

E. DISCUSSION

The infusion of up to 3 litres of water daily into the cecum of pigs each fitted with a single cannula was found to have no effect on N balance and the AD of protein (Just et al. 1979). Thus in the present study, it was not expected that infusion of water (400 ml/d) would afffect N balance and the AD of N and AA.

Both MBM and SBM are protein sources commonly used to complement cereal grains in pig diets in North America. As in previous studies (e.g., Aherne et al. 1979), the present experiment showed the protein quality in MBM to be inferior to that of SBM, as exemplefied both by lower levels of most indispensable AA (Table II.3) and lower AD of indispensable and dispensable AA (Tables II.5 and II.6, respectively). As a consequence, reduced amounts of AA were available for absorption and subsequent utilization for protein synthesis, resulting in lower N retention when pigs were fed MBM (23.8%) than with SBM (50.3%), Table II.4.

The large differences (percentage units) observed between the AD of N (crude protein) and those of

indispensable AA (arginine +11.2, and isoleucine -5.2, in MBM; arginine +7.6, and methionine -7.6, in SBM) suggested that the precise formulation of practical pig diets could not be based on AA digestibility values calculated from the AD of crude protein and the AA analysis of the protein supplement (Just 1979). Rather, the digestibility of the individual dietary AA must be considered (Zebrowska 1978, Tanksley and Knabe 1980).

The role of the hindgut microflora in the degradation of undigested protein residues and the resulting effect on the validity of the fecal analysis method have been studied in experiments with pigs fitted with cannulas at the end of the small intestine. Most workers (Sauer 1976, Sauer et al. 1977a,b, 1979, Zebrowska and Buraczewski 1977, Tanksley and Knabe 1980) found AA digestibilities to be higher when measured in the feces than at the terminal ileum, indicating net AA disappearance in the hindgut. Just (1979) also made similar observations, but reported differences were of smaller magnitude. In contrast, others (Holmes et al. 1974, Sauer et al. 1980) have reported greater ileal than fecal digestibilities for certain AA, indicating net synthesis of AA in the hindgut. These latter findings are substantiated by results of the present study (Table II.8). Only one report (Poppe and Meier 1977) indicated no differences between ileal and fecal AA digestibilities, and attributed the differences reported by the other researchers to technical problems relevant to the location of the ileal

cannula.

The digestibility of N measured at the terminal ileum of pigs fed MBM and SBM diets was shown to be 61.7 and 72.4%, respectively (Zebrowska and Buraczewski 1977), and that of cornstarch 98.2% (Sauer et al. 1977a). In the current experiment, therefore, the approximate daily amounts of protein residues (undigested dietary plus endogenous) expected to enter the hindgut of each pig fed the MBM and SBM diets were 85 and 59 g, respectively. About 18 and 218 g total starch would be present in the hindgut of the water- and starch-infused pigs, respectively. The increased amount of energy relative to protein (40.2 and 58.0 vs 3.3 and 4.8 KJ/g in the starch- vs water-infused pigs fed MBM and SBM, respectively) was expected to markedly enhance microbial activity (Mason and Palmer 1973, Mason et al. 1976). This was demonstrated by increased AA synthesis in the hindgut (Table II.8), and thus increased fecal N excretion, and concomitantly decreased total urinary N excretion.

A possible explanation for the changes that occurred in the route of N excretion following starch infusion is that ammonia, the major N end product of protein digestion in the hindgut (Zebrowska 1975, Hodgdon 1977, Hodgdon et al. 1977), instead of being absorbed through the hindgut, was being used for de novo synthesis of microbial protein voided in the feces. As a result, there was increased output of fecal N and AA, which was reflected in decreases in the AD (percentage units) of N (3.5), indispensable AA (5.0) and

dispensable AA (6.2). Since less ammonia was available in the hindgut, there was a reduction in the amount absorbed into the blood (Hodgdon 1977, Hodgdon et al. 1977), and consequently a decrease in excretion of urinary urea N and total urinary N (Table II.4). It was likely that starch infusion, by decreasing ammonia absorption from the hindgut into the blood, might result in a saving of metabolic energy to the animal. Less ammonia would become available for amination, transamination and conversion into urea, all of which processes require expenditure of metabolic energy (Lehninger 1975). Furthermore, ammonia from recycled urea (P.A. Thacker and J.P. Bowland 1981, Pers. Comm.) would become available in the hindgut. With energy no longer limiting for microbial activity, additional ammonia would be incorporated into microbial protein voided in the feces (Mason and Palmer 1973, Mason et al. 1976, Mendez-Pereira et al. 1977). Therefore, the results of the current study may explain observed increases in the levels of AA between the terminal ileum and the feces, when diets of relatively low digestibility were fed to pigs fitted with ileocecal reentrant cannulas (Sauer et al. 1977b, 1980).

The interaction of protein source x infusion for total urinary N, as well as for the AD of aspartic acid and serine, indicated that bacterial proteolysis, deamination and de novo protein synthesis occurred to a greater extent when the pigs were fed the poorly digestible MBM rather than SBM, and infused with starch rather than water. More protein

residues and starch were available in the hindgut of the starch-infused pigs fed MBM. Also, it was likely that, because of higher blood urea N in the pigs fed the poor quality protein from MBM relative to SBM (Brown and Cline 1974), more blood urea would be secreted into the digestive tract (Mosenthin 1981), resulting in further ammonia incorporation into microbial protein. In the present study when MBM was fed, starch infusion increased the fecal AA excretion (g/d) from 4.3 to 5.7 for aspartic acid, and 2.0 to 2.6 for serine; when SBM was fed, corresponding increases were smaller, i.e., 3.0 to 3.3 and 1.3 to 1.4, respectively (Table II.8).

The observation that the AD of individual AA were reduced by starch infusion clearly indicated that when a given protein supplement is fed in a mixed diet, the digestibility of the starch at the end of the small intestine, and therefore the amount entering the hindgut, must be taken into account (Mason and Palmer 1973, Mason et al. 1976). Ignoring this would lead to two types of errors. First, AA availabilities (measured by AD, and reflecting the amounts of AA available for absorption) might be overestimated (Zebrowska 1975, Sauer et al. 1977a,b, 1979,1980, Zewbrowska and Buraczewski 1977). This occurs when a highly digestible starch — e.g., cornstarch — is fed (Mason and 1973, Mason et al. 1976) and was simulated by water infusion in the present study. Microbial proteolysis is followed by deamination (disappearance) of AA. Ammonia is

absorbed from the hindgut into the hepatic portal blood (Hodgdon 1977, Hodgdon et al. 1977), and excreted in the urine, mainly as urea (Zebrowska 1975, Just et al. 1979). The second possible form of error is that AA availabilities might be underestimated. This occurs when a poorly digestible starch - e.g., potato starch - is fed (Mason and Palmer 1973, Mason et al. 1976), and was simulated in this study by starch infusion. In this latter case, the ammonia is utilized by the microflora for the synthesis of AA, which are excreted in the feces. The latter error tends to be magnified when a protein of lower digestibility (e.g., MBM as opposed to SBM) is fed in a diet containing a poorly digestible source of energy.

The present results showed that the excretion of N via the feces and the urine was affected by the infusion of starch at the terminal ileum. The amount of starch (fermentable energy substrate) entering the hindgut determined the extent of microbial degradation of undigested protein residues and subsequent synthesis of microbial AA to be voided in the feces, thereby increasing fecal N and AA excretion, and consequently decreasing AD measurements. At the same time, there was a decrease in urinary N excretion. Starch infusion had no effect on the amount of N retained by the pigs. In conclusion, it is clear that the source of carbohydrate included in the diet is one of the factors that determine the AD of N and AA of a given protein supplement.

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III. NITROGEN AND AMINO ACID METABOLISM IN THE HINDGUT OF PIGS FED BARLEY OR WHEAT DIETS AS AFFECTED BY THE INFUSION OF CORNSTARCH AT THE TERMINAL ILEUM[2]

A. ABSTRACT

Growing pigs (40 to 45 kg liveweight) were each surgically fitted with a single T-shaped cannula at the end of the small intestine, and fed diets consisting of barley or wheat which served as sole sources of both energy and protein. Cornstarch (CS, 200 g/d) or water (400 g/d) was infused through the cannulas and the effects on the metabolism of nitrogen (N) and amino acid (AA) in the hindgut were studied. Infusion of CS, as opposed to water, increased excretion of fecal N ($P<0.05$) but decreased excretion of total urinary N ($P<0.01$). There was no effect ($P>0.05$) on the amount of N retained or the urinary urea N content. In addition, starch infusion significantly decreased the apparent digestibilities (percentage units) of N (6.1); indispensable AA: lysine (14.4), threonine (8.5), isoleucine (8.4) and histidine (6.2); and dispensable AA: alanine (13.2), aspartic acid (10.5), tyrosine (8.7) and

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glycine (8.0). The same pattern of digestibility decreases was observed for both diets, but in general the magnitude of these decreases tended to be greater for barley than for wheat. These decreases could be attributed to the apparent synthesis of AA in the hindgut. Results indicated that the route of N excretion as well as the proportions of N excreted via feces and urine were affected by the amount of CS (energy substrate) entering the hindgut. When energy was limiting (water infusion), undigested protein was degraded by the hindgut microbes and the resulting N end products excreted largely in the urine. When energy was not limiting (CS infusion), the microbes utilized N end products of protein degradation for de novo synthesis of AA (proteins) which were excreted in the feces. In the former case, therefore, apparent AA digestibilities, measured by the fecal analysis method, were overestimated; in the latter case, these measures were underestimated. In determinating the AA availabilities from protein sources, the effects of the hindgut microflora on N and AA metabolism must be considered.

B. INTRODUCTION

For optimal growth and performance, pigs require balanced diets which provide adequate levels of all nutrients, including energy and AA. As commonly used ingredients of pig diets, cereal grains serve as major sources of both energy and AA. Requirements for additional

AA are usually met by supplementing the diets with one or more protein-rich feed ingredients such as fish meal, meat-and-bone meal, canola meal and soybean meal (NRC 1979)

In the previous experiment (Chap. II), growing pigs were fed a diet containing CS (of high ileal digestibility) supplemented with meat-and-bone meal or soybean meal as sole sources of protein. Additional starch was made available to the microflora of the hindgut via infusion at the terminal ileum. The results indicated that the metabolism of N and AA in the hindgut was influenced by the presence of starch as an energy substrate for microbial activity. Starch infusion changed the pathways of N excretion, as was shown by an increased excretion of fecal N and a concomitant decrease in total urinary N, including urinary urea N. There was no effect on the amount of N retained. The increase in the excretion of fecal N and AA resulted in marked decreases in their apparent digestibilties. These decreases were more pronounced when the protein source was meat-and-bone meal rather than soybean meal which has a higher apparent digestibility of N, measured at the end of the small intestine. Clearly, these results cast doubt on the validity of the fecal analysis method (Kuiken and Lyman 1948) which takes into account only the amounts of individual nutrients (e.g., crude protein and AA) ingested in the diet and excreted in the feces, while ignoring the metabolic processes in the hindgut. An underlying assumption of this method is that there is no transformation (synthesis or

degradation) of residual dietary protein and AA in the hindgut arising from microbial action. However, several workers have reported net degradation and disappearance of AA (Cho and Bayley 1972, Zebrowska 1973, 1975, 1978, Mason et al. 1976, Hodgdon et al. 1977, Sauer et al. 1977a,b, 1979, 1980, Zebrowska and Buraczewski 1977, Just et al. 1979, Low 1979, Tanksley and Knabe 1980) or net synthesis (Cho and Bayley 1972, Holmes et al. 1974, Mason et al. 1976, Sauer et al. 1977b, 1980, Low 1979, Sauer and Just 1979, Just et al. 1980). The extent of degradation or synthesis of individual AA seems to be dietary dependent, with fibre level (Sauer and Just 1979, Just et al. 1980, Sauer et al. 1980), protein source (Carlson and Bayley 1970, Cho and Bayley 1972, Hodgdon et al. 1977, Zebrowska and Buraczewski 1977, Zebrowska 1978, Tanksley and Knabe 1980, Misir and Sauer 1980, 1981a), starch type (Mason and Palmer 1973, Mason et al. 1976) and starch pretreatment (Livingstone et al. 1977), being important determinants. Therefore, the fecal analysis method may overestimate or underestimate the amounts of AA actually available for absorption and subsequent protein synthesis by the pig.

The objective of the present investigation was to study the effects of CS infusion at the terminal ileum on the metabolism of N and AA in the hindgut of pigs fed diets containing barley or wheat. Apart from being sources of energy, these cereals also provided the only source of AA to the pigs.

C. MATERIALS AND METHODS

Six Yorkshire x Lacombe barrows, ranging in initial body weight from 40 to 45 kg, were each surgically fitted with a single T-shaped cannula at the end of the small intestine, approximately 5 cm from the ileocecal junction. Surgical procedures, cannula design, postsurgery care and management of the cannulated animals before being put on test, room conditions, metabolic cages used to house the animals, and procedures for sample collections have been described (Chap. II).

Each of the experimental periods (n=2) consisted of a 5-d adaptation period followed immediately by a 5-d collection period. At the commencement of the experiment, pigs (n=3) were randomly selected and fed diets (n=2) containing the same percentage barley or wheat. Vitamins and minerals were added at or above NRC (1979) specifications (Table III.1). Prior to being mixed with the other ingredients, the grain samples were ground to pass through a 2 mm screen. To facilitate pelleting, water (5% w/w) was added to each complete diet which was then thoroughly mixed in a Hobart food mixer (Toronto, Ontario, Canada). The diets were then pelleted (without steam) by means of a Superior Templewood machine (Hopkins, Minnesota, USA) equipped with a 5 mm die. Thereafter, the pelleted diets were spread thinly (1 cm) to air-dry on a concrete floor.

Each pig was given 800 g diet twice daily, at 0800 h and 1600 h throughout the experiment. Water was provided ad

libitum. Within a period of 1 h following feeding, infusions (n=2) of water or a slurry of CS (100 g/d) was administered gradually through the cannula of each pig, using a 50 ml catheter-tip syringe. The infused CS provided 15.2 MJ of gross energy. The infusion pattern followed in period 1 was reversed in period 2 (Chap. II, Table II.2). The average initial and final weights of the pigs during the experiment were 54 and 60 kg, respectively.

Chemical Analyses

Samples of diets and composite freeze-dried feces were ground to pass through a 0.8 mm screen prior to analyses. The diets were analyzed for dry matter, Kjeldahl N and gross energy; feces for dry matter and Kjeldahl N. A sample of CS used in the infusions was analyzed for gross energy; urine samples from each pig (pooled among days for each period) for Kjeldahl N (AOAC 1970), and also for urea N, using an autoanalyzer (Anonymous 1974). Furthermore, duplicate feed samples and single fecal samples for all the pigs during both periods were analyzed for AA content as described (Sarwar and Bowland 1975).

Calculations and Statistical Analyses

Total feed intake, N balance and apparent digestibilities (AD) of dry matter, crude protein and individual AA, were calculated as described (Chap. II).

The data for each response criterion were subjected to least squares analysis of varianace (Mehlenbacher 1978). Differences among treatment means were determined by the

Student-Newman-Keuls' multiple range test (Snedecor and Cochran 1967).

D. RESULTS

Both barley and wheat diets had similar levels of gross energy, i.e., 16.60 and 16.51 MJ/Kg, respectively (Table III.1). Analyses of the diets showed lower levels of most AA in the barley than in the wheat diet; however, the levels of lysine and threonine were similar (Table III.2). Daily feed dry matter consumption was similar for both diets (1425 and 1419 g for the barley and wheat diets, respectively); however, the daily intake of N (Table III.3) was lower for the barley-fed pigs (27.0 g) as compared to the wheat-fed pigs (36.1 g), reflecting the different N contents of the two diets (Table III.1).

As a percentage of the respective N intakes, pigs fed the barley as compared to the wheat diet excreted more N in the feces ($P<0.01$); however, those fed the wheat diet excreted greater amounts of total urinary N ($P<0.01$), including urinary urea N ($P<0.01$). There was no difference ($P>0.05$) in the amount of N retained (Table III.3). In addition for the barley diet, there were lower AD for dry matter ($P<0.01$), crude protein ($P<0.01$) and indispensable AA: arginine ($P<0.01$), histidine ($P<0.01$), isoleucine ($P<0.05$), leucine ($P<0.01$), lysine ($P<0.05$), phenylalanine ($P<0.01$), threonine ($P<0.01$) and valine ($P<0.01$), Table III.4; and dispensable AA: alanine ($P<0.01$), aspartic acid

Table III.1. Composition and partial chemical analyses of the basal diets.

Diets:	Barley	Wheat
Ingredients (%, as fed)		
Barley (11.3% CP)	95.28	-
Wheat (14.9% CP)	-	95.28
Tallow	2.00	2.00
Calcium carbonate (38% Ca)	1.25	1.25
Calcium phosphate (17% Ca, 21% P)	0.75	0.75
Trace mineralized salt[1]	0.50	0.50
Trace mineral premix[2]	0.15	0.15
Vitamin premix[3]	0.015	0.015
Choline chloride	0.055	0.055
Analyses (as fed basis)[4]		
Dry matter (%)	90.62±0.25	89.11±0.21
Nitrogen (%)	1.72±0.01	2.27±0.02
Gross energy (MJ/kg)	16.60±0.03	16.51±0.03

[1,2,3]Source and composition of the trace mineralized salt, trace mineral and vitamin premixes were the same as described, Table II. I, footnotes 1 - 3.

[4]Mean ± standard error.

Table III.2. Partial amino acid composition of the diets.

Diets:	Barley	Wheat
Amino acids, % dry matter[1]		
Indispensable		
Arginine	0.57	0.68
Histidine	0.25	0.36
Isoleucine	0.44	0.55
Leucine	0.84	1.01
Lysine	0.43	0.40
Methionine	0.21	0.26
Phenylalanine	0.63	0.76
Threonine	0.43	0.45
Valine	0.62	0.66
Dispensable		
Alanine	0.46	0.51
Aspartic acid	0.71	0.72
Cysteine	0.14	0.18
Glutamic acid	2.92	5.02
Glycine	0.45	0.58
Proline	1.37	1.79
Serine	0.50	0.68
Tyrosine	0.25	0.32

[1]Average of two analyses.

Table III.3. Effect of grain and infusion at the terminal ileum on nitrogen balance and urinary urea nitrogen in pigs.

Factor	Intake N	Fecal N	Urinary N	Retained N	Urinary urea N
	—g/5d—	—	% of Intake N	—	—
Grain (G)					
Barley	135.1	28.2[A2]	38.3[B]	33.5	11.6[B]
Wheat	180.7	14.5[B]	57.9[A]	27.6	22.4[A]
SEM[1]	-	1.16	1.84	1.98	0.74
Infusion (I)					
H_2O	158.2	18.3[b2]	51.5[A]	30.1	17.8
Cornstarch (CS)	157.6	24.4[a]	44.6[B]	30.9	16.3
SEM	-	0.91	0.32	1.05	0.43
G x I					
Barley x H_2O	135.2	24.8	41.0	34.2	12.5
Barley x CS	134.9	31.6	35.5	32.8	10.8
Wheat x H_2O	181.1	11.8	62.1	26.1	23.0
Wheat x CS	180.3	17.2	53.7	29.0	21.8
SEM	-	1.31	0.46	1.50	0.61

[1]Standard error of the mean.

[2]For each factor, means within a given column not followed by the same letter are significantly different: A,B at $P<0.01$; a,b at $P<0.05$.

($P<0.01$), glutamic acid ($P<0.01$), glycine ($P<0.01$), proline ($P<0.01$), serine ($P<0.01$) and tyrosine ($P<0.05$), Table III.5. For both grains the digestibilities of individual AA were markedly higher or lower than that of the respective N (crude protein). For the indispensable AA, the values (percentage units) ranged from -16.6 (lysine) to +8.7 (phenylalanine) for barley, and from -16.1 (lysine) to +5.0 (phenylalanine) for wheat (Table III.4); for the dispensable AA the range differences were larger, i.e., from -13.2 (alanine) to +17.3 (proline) for barley, and from -9.2 (aspartic acid) to +10.9 (proline) for wheat (Table III.5).

The infusion of CS, as opposed to water, increased the excretion of fecal N ($P<0.05$) and concomitantly decreased total urinary N ($P<0.01$). There was no effect ($P>0.05$) on the amount of N retained or the content of urea N in the urine (Table III.3). Furthermore, CS infusion decreased the AD of dry matter ($P<0.01$), N ($P<0.05$), the indispensable AA: arginine ($P<0.05$), histidine ($P<0.05$), isoleucine ($P<0.05$), lysine ($P<0.05$), phenylalanine ($P<0.05$) and threonine ($P<0.01$), Table III.4; and the dispensable AA: alanine ($P<0.05$), aspartic acid ($P<0.01$), glutamic acid ($P<0.05$), glycine ($P<0.05$), proline ($P<0.05$), serine ($P<0.01$) and tyrosine ($P<0.05$), Table III.5. For the indispensable AA, the largest significant decreases (percentage units) were observed for lysine (14.4), threonine (8.5), isoleucine (8.4) and histidine (6.2), whereas for the dispensable AA, the largest decreases were for alanine (13.2), aspartic acid

Table III.4. Effect of grain and infusion at the terminal ileum on apparent digestibility of dry matter, nitrogen and indispensable amino acids (%).

Factor	DM[1]	N	Arg	His	Ile	Leu	Lys	Met	Phe	Thr	Val
Grain (G)											
Barley	82.5^{B}[2]	71.8^{B}	79.1^{B}	79.1^{B}	70.9^{b}[2]	76.9^{B}	55.2^{b}	71.3	80.5^{B}	67.7^{B}	69.6^{B}
Wheat	87.5^{A}	85.5^{A}	89.6^{A}	89.1^{A}	84.0^{a}	90.0^{A}	69.4^{a}	82.2	90.5^{A}	80.8^{A}	85.0^{A}
SEM[3]	0.29	1.15	0.60	1.03	2.09	0.69	2.63	3.28	0.59	1.24	1.03
Infusion (I)											
H_2O	86.5^{A}	81.7^{a}	87.3^{a}	87.2^{a}	81.6^{a}	85.7	69.5^{a}	81.9	87.9^{a}	78.5^{A}	80.1
CS[4]	83.5^{B}	75.6^{b}	81.4^{b}	81.0^{b}	73.2^{b}	81.2	55.1^{b}	71.6	83.1^{b}	70.0^{B}	74.6
SEM	0.35	0.91	0.81	0.92	1.66	1.32	2.18	3.32	0.67	0.91	2.03
G x I											
Barley x H_2O	84.6	75.2	83.4	83.5	75.9	80.9	63.5	76.1	83.9	73.3	74.6
Barley x CS	80.4	68.4	74.8	74.7	65.9	73.0	46.9	66.5	77.1	62.2	64.5
Wheat x H_2O	88.4	88.2	91.3	90.8	87.3	90.6	75.5	87.7	91.8	83.8	85.5
Wheat x CS	86.6	82.8	87.9	87.4	80.6	89.5	63.2	76.7	89.1	77.8	84.6
SEM	0.50	1.31	1.16	1.33	2.38	1.90	3.12	4.76	0.97	1.31	2.91

[1]Dry matter. The infused starch was considered as dry matter intake for calculation of DM digestibilities.

[2]For each factor, means within a given column not followed by the same letter are significantly different: A,B at $P<0.01$; a,b at $P<0.05$.

[3]Standard error of the mean.

[4]Cornstarch.

Table III.5. Effect of grain and infusion at the terminal ileum on apparent digestibility of dispensable amino acids (%).

Factor	Ala	Asp	Glu	Gly	Pro	Ser	Tyr
Grain (G)							
Barley	58.6^{B1}	61.8^{B}	87.2^{B}	67.8^{B}	89.1^{B}	76.9^{B}	71.3^{b1}
Wheat	77.6^{A}	76.3^{A}	95.9^{A}	84.4^{A}	96.4^{A}	89.4^{A}	83.5^{a}
SEM[2]	2.41	1.65	0.48	1.16	0.34	0.72	2.05
Infusion (I)							
H_2O	74.7^{a}	74.3^{A}	93.1^{a}	80.1^{a}	94.0^{a}	86.0^{A}	81.7^{a}
CS[3]	61.5^{b}	63.8^{B}	90.0^{b}	72.1^{b}	91.5^{b}	80.3^{B}	73.0^{b}
SEM	1.94	1.27	0.44	1.16	0.39	0.61	1.89
G x I							
Barley x H_2O	67.2	68.3	89.5	73.6	91.1	81.1	76.2
Barley x CS	50.1	55.3	84.9	62.0	87.1	72.8	66.3
Wheat x H_2O	82.1	80.3	96.7	86.6	96.9	91.0	87.1
Wheat x CS	73.0	72.3	95.0	82.2	95.8	87.9	79.8
SEM	2.79	1.83	0.64	1.66	0.56	0.88	2.72

[1]For each factor, means within a given column not followed by the same letter are significantly different: A,B at $P<0.01$; a,b at $P<0.05$.

[2]Standard error of the mean.

[3]Cornstarch.

(10.5), tyrosine (8.7) and glycine (8.0).

There was no grain x infusion interaction for any of the parameters including fecal N, total urinary N and urinary urea N, retained N, and AD of dry matter, crude protein and all AA, indicating a similar pattern of response to CS infusion when either grain was fed (Tables III.3 to 5). In general, however, the magnitude of the responses, and especially AD values, tended to be more pronounced when barley rather than wheat was fed.

There was no difference in the content of N and levels of AA, except proline ($P<0.05$), in the feces produced by both the barley- and wheat-fed pigs. Infused CS had no effect ($P>0.05$) on fecal AA composition (Table III.6), suggesting quantitative rather than qualitative effects of energy infusion on AA metabolism in the hindgut.

The apparent increase (net synthesis) of AA in the hindgut attributable to CS infusion showed great variation (Table III.7). For the indispensable AA, the values (g/d) ranged from 0.26 (methionine) to 0.92 (lysine) for barley, and 0.08 (valine) to 0.63 (lysine) for wheat; for the dispensable AA, values ranged from 0.32 (tyrosine) to 1.74 (glutamic acid) for barley, and 0.25 (proline) to 1.08 (glutamic acid) for wheat.

Table III.6. Effect of infusion at the terminal ileum on feces composition of pigs fed barley or wheat diets.

Diet:	Barley		Wheat		
Infusion:	H_2O	Starch	H_2O	Starch	SEM[1]
Amino acids, g/16 g N					
Indispensable					
Arginine	3.35	3.87	3.14	3.09	0.25
Histidine	1.49	1.72	1.70	1.67	0.15
Isoleucine	3.70	3.95	3.48	3.32	0.27
Leucine	5.56	6.09	5.09	5.11	0.39
Lysine	5.54	6.09	5.14	5.58	0.41
Methionine	1.79	1.88	1.61	1.67	0.14
Phenylalanine	3.57	3.86	3.20	3.09	0.23
Threonine	3.97	4.30	3.79	3.71	0.26
Valine	5.39	5.86	5.10	5.01	0.39
Dispensable					
Alanine	5.39	6.18	4.67	5.18	0.47
Aspartic acid	7.87	8.45	7.42	7.53	0.49
Glutamic acid	10.71[ab2]	11.76[a]	8.73[b]	9.26[ab]	0.63
Glycine	4.21	4.57	4.01	3.91	0.35
Proline	4.30[a]	4.68[a]	2.76[b]	2.85[b]	0.38
Serine	3.30	3.61	3.17	3.09	0.24
Tyrosine	2.17	2.32	2.13	2.04	0.18
Nitrogen, %[3]	2.98	3.07	2.41	3.07	-
Amino acid N recovered, %[3]	72.31	79.37	65.49	66.11	-

[1]Standard error of the mean.

[2]For each amino acid, means not followed by a common superscript are significantly different at $P<0.05$.

[3]Average of 3 pigs per infusion (H_2O versus starch) for each diet.

Table III.7. Effect of cornstarch infusion at the terminal ileum on the apparent increase in amino acid content in the feces.

Diets:	Barley	Wheat
Amino acid, g/d[1]		
Indispensable		
Arginine	0.64	0.29
Histidine	0.29	0.15
Isoleucine	0.57	0.47
Leucine	0.86	0.14
Lysine	0.92	0.63
Methionine	0.26	0.36
Phenylalanine	0.55	0.26
Threonine	0.62	0.34
Valine	0.81	0.08
Dispensable		
Alanine	1.02	0.59
Aspartic acid	1.19	0.74
Glutamic acid	1.74	1.08
Glycine	0.68	0.32
Proline	0.71	0.25
Serine	0.53	0.27
Tyrosine	0.32	0.30

[1]Average of three pigs per infusion (H_2O versus starch) for each diet.

E. DISCUSSION

A clear understanding of the factors influencing the metabolism of N and AA in the hindgut of the pig would permit more precise estimates of the digestibilities (and therefore availabilities) of the constituent AA in any given dietary protein ingredient. Such data are a prerequisite to capitalizing on the full potential nutritional value of a protein ingredient since a diet may be adequate in total crude protein (NRC 1979) but still does not provide the pig with all the AA required for optimal protein synthesis (Zebrowska 1978, Tanksley and Knabe 1980).

The infusion at feeding time of 800 ml of water via cannulas at the terminal ileum of pigs was shown to have no effect on N-balance and apparent N digestibility (R. Misir and W.C. Sauer, Unpubl.). Therefore, in the present study, water infusion was assumed to have no effect on the parameters measured.

In diets containing 100% barley or wheat (Sauer et al. 1974), or either grain supplemented with only vitamins and minerals (Sauer et al. 1977b, 1979), the AD of N and AA were found to be lower for barley than for wheat. In the current study, a similar trend was observed for the barley or wheat diets (water infusion); however, the wide variations in AD of individual AA from that of the respective N (crude protein) clearly suggested that the digestibility of individual AA, and not that of the crude protein (Just 1979), must be taken into account when formulating diets.

This is especially important in those diets containing predominantly cereal grains which are usually limiting in lysine and threonine (Munck et al. 1970). Also, among the indispensable AA in cereal grains, the digestibility of lysine is usually lowest (Sauer 1976, Eggum 1977, Misir and Sauer 1981c).

The metabolism of N and AA in the hindgut is a reflection of the extent of microbial activity and invariably is manifested by degradation of undigested protein residues and subsequent de novo synthesis of bacterial protein. Net disappearance or synthesis of N compounds in the hindgut is dependent on the amount of fermentable energy substrates such as starch (Mason et al. 1976) and fibre (Sauer and Just 1979, Sauer et al. 1980) entering the hindgut.

Various researchers (Sauer et al 1977a,b, Zebrowska 1978 and Tanksley and Knabe 1980) have reported higher values for AA digestibilities, measured by the fecal analysis method (Kuiken and Lyman 1948), in contrast to digestibilties determined at the terminal ileum. Previous studies provided data on chemical analyses for crude protein, starch and crude fibre in barley and wheat diets. The apparent ileal digestibilities of crude protein and starch (Sauer et al. 1977a) and crude fibre (Sauer 1976) in pigs were found to be 74.9, 92.0 and 5.0% for barley, and 82.9, 93.1 and 0.0% for wheat, respectively. In the current experiment, therefore, the amount of crude protein

(undigested dietary plus endogenous), residual starch and crude fibre expected to enter the hindgut were approximately 39, 65 and 64 g or 34, 57 and 31 g for the barley- or wheat-fed pigs, respectively. The infusion of CS at the terminal ileum increased by 200 g/d the amount of energy available to the hindgut microflora. The resulting increase in the ratio of total starch to protein in the barley- or wheat-fed pigs (8.4 or 8.5 vs 3.3 or 2.6 g/g for CS vs water infusion) was expected to greatly enhance microbial activity (Misir and Sauer 1981a). With energy no longer limiting, microbial proliferation would result in alteration in the metabolism of N and AA. This was demonstrated in the current study by increased output of fecal N and concomitant decrease in the excretion of total urinary N, as was also observed previously (Mason et al. 1977, Misir and Sauer 1981a). This observation could be explained by the finding that ammonia, the major N end product of protein digestion in the hindgut (Hodgdon et al. 1977), instead of being absorbed into the blood, was utilized for de novo synthesis of microbial protein voided in the feces, thereby altering the route of N excretion.

The effect of CS infusion on decreases in the apparent fecal digestibilities was most pronounced for the poorly digestible AA including the indispensable AA: lysine, threonine and isoleucine (Table III.4), and the dispensable AA: alanine, aspartic acid, tyrosine and glycine (Table III.5); in contrast, for the highly digestible AA, the

decreases were much smaller. In some cases, however, it might be more meaningful to consider the AD reductions in absolute rather than in relative terms. This is exemplified by threonine and glutamic acid whose AD were decreased by 8.5 and 3.1 percentage units, whereas the corresponding values (g/d) were 0.48 and 1.41, respectively.

The greater net amount of AA apparently synthesized in the hindgut of pigs fed barley (11.71 g/d) as compared to those fed wheat (6.27 g/d) might be related to the lower ileal digestibilities of barley crude protein and constituent AA (Sauer et al. 1977a). Since the ratio of energy to protein in the hindgut was similar for both the barley- and the wheat-fed pigs, the extent of AA synthesis and therefore fecal AD of AA, might be related to the amount of protein residues entering the hindgut (Misir and Sauer 1981a).

As indicated earlier in this chapter, previous data on barley and wheat have shown the AD of AA, measured at the terminal ileum, to be lower than AD values taken over the total intestinal tract. Because CS infusion depressed fecal AA digestibilties, as also noted in this study, it was obvious that the fecal AA digestibility was dependent on the amount of starch available to the hindgut microbes (Misir and Sauer 1981a, Mason and Palmer 1973, Mason et al. 1976, 1977). Thus, in the presence of adequate starch (fermentable energy substrate) the apparent fecal digestibility may not be different from apparent ileal digestibility. Conceivably

in such a situation, the fecal analysis method might provide reliable estimates of AA availabilities. The results of the present experiment could therefore explain data (Sauer et al. 1980) which showed little or no difference between ileal and fecal digestibilities of AA in barley-based diets supplemented with 6 to 9% crude fibre (provided by barley straw or wheat bran). There were larger differences in AA digestibilities at lower levels of crude fibre.

Frequently, digestibility experiments are conducted to determine the availability of the AA in a given protein supplement (Sauer et al. 1977a,b, Zebrowska and Buraczewski 1977, Tanksley and Knabe 1980). Results obtained are used in formulation of pig diets. In practical cereal-based diets, it is generally assumed that the contribution of the individual protein sources are linear and therefore additive (NRC 1979). The present study has shown that in evaluating the nutritive value of protein sources in a diet where cereal grains are the major energy ingredients, consideration must be given to the associative effects of energy and protein, particularly with regard to the increased microbial synthesis of AA and consequent decreases in estimates of apparent fecal digestibilities of AA. Furthermore, since disappearance of AA in the hindgut is not synonymous with AA absorption (Zebrowska 1973, 1975, Hodgdon et al. 1977) it would seem that ileal digestibilities are more valid than those values determined over the total digestive tract.

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IV. EFFECT OF CARBOHYDRATE AND PROTEIN INFUSION AT THE TERMINAL ILEUM ON NITROGEN METABOLISM IN THE HINDGUT OF THE GROWING PIG[3]

A. ABSTRACT

Eight growing pigs, initial liveweight 28 to 32 kg, were each fitted with a single T-shaped cannula at the end of the small intestine, and fed a cornstarch (CS)-soyprotein (SP) diet formulated to 13% crude protein. The effects of purified and natural carbohydrates (CHO), protein, and protein plus CHO, infused through the cannula, on nitrogen (N) metabolism in the hindgut were evaluated in two separate 4 x 4 Latin Square experiments. The following treatments (infusions) were administered within 1 h of feeding at 0800 and 1600 h daily: water (200 ml), a CS slurry (100 g/200 ml), pectin (PC) gel (52 g/600 ml), or wheat bran (WB) suspension (88 g/500 ml) in experiment 1; and water (200 ml), and slurries of CS (100 g/200 ml), SP (24 g/200 ml) or CS + SP (100 + 24 g, respectively / 200 ml) in experiment 2. Infused CS or WB provided 3.06, and PC 1.53 MJ gross energy/d. Total feces and urine were collected during the last 5 d of each 10-d test period. In experiment 1, infusion of CS, PC, and WB to a greater extent, increased ($P<0.05$)

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the percentage output of fecal N (FN); the percentage urinary N (UN) was decreased ($P<0.05$) by CS or WB infusion. In experiment 2, the percentage FN was increased ($P<0.05$) and UN decreased ($P<0.05$) by infusion of CS or CS + SP; infused SP had no effect ($P>0.05$) on FN output. In both experiments, excretion of total UN including urea N, was increased ($P<0.05$) by infusion of protein or protein plus CHO substrates, i.e., WB, SP or CS + SP, respectively. Results suggested that endogenous N was entering the hindgut. Furthermore, the pathway of N excretion was dependent on the amount of both energy and N substrates (undigested and endogenous) entering hindgut. Infused SP was completely digested and absorbed in the hindgut. As measured by N retention and apparent biological value, absorbed N did not contribute to the N status of the pig but was excreted mainly as urea in the urine. Values obtained for apparent digestibility (AD) of protein (fecal analysis method) were dependent on the amount of CHO infused into the hindgut.

B. INTRODUCTION

The digestion of dietary protein and energy ingredients in the hindgut of pigs has been investigated using normal animals in slaughter experiments (Cho and Bayley 1972, Mason et al. 1976), and animals fitted with cannulas at the terminal ileum (Zebrowska 1973, 1975, Sauer et al. 1977a,b, Gargallo and Zimmerman 1981, Misir and Sauer 1980, 1981a,b). Accumulated evidence suggests that both nitrogenous and

energy residues entering the hindgut are degraded by the resident microflora (Sauer et al. 1977a,b, Misir and Sauer 1980, 1981a,b, Just et al. 1980, 1981, Alimon and Farrell 1980, Gargallo and Zimmerman 1981). The extent of hindgut digestion is dependent on the composition of the diet, and is known to be influenced by protein type (Carlson and Bayley 1970, Misir and Sauer 1980, 1981a), starch type (Cunningham et al. 1963, Mason et al. 1976), and fibre level (Sauer et al. 1980). The carbon moiety of these substrates are converted to volatile fatty acids (Argenzio and Southworth 1974, Imoto and Namioka 1978, Kennelly et al. 1981) and contributes to the animal's energy balance (Friend et al. 1964, Imoto and Namioka 1978, Kennelly et al. 1981); the N moiety may be beneficial, but only at low levels of N intake (Zebrowska et al. 1977).

In practical pig diets, the polysaccharides in cereal grains provide the major energy substrates - i.e., starch, and fibre which consists of cellulose and hemicellulose such as PC. The objective of this study was to evaluate the effects of infusion of different sources of carbohydrates (purified and natural) and protein into the hindgut of growing pigs on N balance, apparent biological value and apparent N digestibility.

C. MATERIALS AND METHODS

Animals and Management

Eight Yorkshire x Lacombe barrows, ranging in initial body weight from 28 to 32 kg, were each surgically fitted with a single T-shaped cannula at the end of the small intestine. Surgical procedures, design of the cannulas, post-surgery care and management of the cannulated pigs before being put on test, room conditions, and procedures for sample collection and storage have been described (Chap. II).

During the experiments, the pigs were fed a semi-purified diet consisting mainly of CS and SP (promine, an isolated soyprotein, Central Soya Co., Inc., Chicago, Ill., USA), and formulated to 13% crude protein (N x 6.25), slightly below the recommended NRC (1979) level for growing pigs. Alpha floc (a purified cellulose product, Lee Chemicals, 1119 Yonge Street, Toronto, Ontario) was included as a dietary diluent at a level of 7% to minimize possible problems during defecation, e.g., rectal prolapse, previously observed when pigs were fed semi-purified type diets (Sauer 1976). Dextrose was added at 10% level to improve palatability; minerals and vitamins to meet or exceed NRC (1979) specifications (Table IV.1).

The barrows were housed in stainless steel metabolic cages which allowed separate collection of feces and urine. At the start of the two experiments (which were run concurrently), the 8 pigs were randomly alloted into groups

Table IV.1. Composition and partial chemical analyses of the basal diet.

Ingredients (as fed)	%
Cornstarch	61.58
Soyprotein (83.3% CP)	14.40
Dextrose	10.00
Alpha floc	7.00
Tallow	4.00
Calcium carbonate (38% Ca)	0.75
Calcium phosphate (17% Ca; 21% P)	1.55
Trace mineralized salt[1]	0.50
Trace mineral premix[2]	0.15
Vitamin premix[3]	0.015
Choline chloride	0.055
Chemical analyses (as fed basis)[4]	
Dry matter	90.44±0.22
Nitrogen	2.08±0.01

[1,2,3]These have been described (Table II.1, footnotes 1-3).

[4]Average of two analyses ± standard error.

of 4 pigs. Each pig was given 800 g diet at 0800 and 1600 h daily throughout the test periods. Within 1 h of feeding, one of four substances or combination of substances (treatments) was gradually infused through the cannula of each pig, using a 50-ml catheter tip syringe. In experiment 1, the treatments consisted of water (200 ml) which served as the control, a slurry of CS (100 g/200 ml), a gel of PC (52 g/600 ml; Sigma Chemical Co., St. Louis, MO, USA) and a suspension of ground (0.8 mm) WB (88 g/500 ml); in experiment 2, water and slurries of CS (as above), SP (24 g/200 ml) and CS plus SP (24 + 100 g, respectively /200 ml). The average initial and final weights of the barrows during the experiments were 40 and 52 kg, respectively.

Each experimental period lasted 10 days. Total feces and urine were collected during the last 5 days, as described (Chap. II).

Calculation of AD, and N balance parameters had been been described (Chap. II). Apparent biological value was taken as the proportion of the apparently absorbed N that was retained.

Analyses

Duplicate analyses were conducted on ground samples (0.8 mm) of SP, the diet, oven-dried (60°C) feces (pooled for each pig within periods), and air dry samples of CS, PC, SP and WB for dry matter and Kjeldahl N (AOAC 1970). In addition, CS, PC and WB were analyzed for gross energy; filtered urine samples (pooled for each pig within periods)

for Kjeldahl N, and urea N using an autoanalyzer (Anonymous 1974).

The effects of the treatments were evaluated in two separate concurrent 4 x 4 Latin Square experiments of barrows x infusions. Differences among treatment means were established by the Student-Newman-Keuls' multiple range test (Snedecor and Cochran 1967).

D. RESULTS AND DISCUSSION

Starch consists of a mixture of straight and branched chains of glucose units, whereas pectins are polymers of methyl-D-galacturonate (Lehninger 1975). In cereal grains (seeds), starch is the major energy reserve; pectins serve as cementing components of cell walls, and may account for 0.56 and 1.34% in barley and wheat, respectively (McNab and Shannon 1974). Wheat bran provides a natural source of both starch and PC, in addition to cellulose and protein. Cereal starches are highly digestible in the small intestine (Keys and DeBarthe 1974b, Sauer et al. 1977a), whereas the fibres are digested mainly by the microbes of the hindgut (Keys and DeBarthe 1974a, Hove and King 1979).

At the start of this study, an attempt was made to infuse 104 g PC twice daily so as to provide the microbes of the hindgut with the same amount of gross energy as the infused CS or WB, i.e., 3.04 MJ/d; however, this plan had to be abandoned because of the large volume of water (>1.2 l) required to make a gel suitable for infusion into one pig.

The PC for infusion was prepared daily about 5 min before feeding time by adding 52 g to 600 ml water at 37°C with constant stirring until a homogenous gel was obtained. Infused CS and PC served as energy substrates for the hindgut microbes; WB provided both energy and protein, and SP mainly protein (83.3%).

The influence of water infusion on N balance and apparent N digestibility had been studied in an experiment (R. Misir and W.C. Sauer, Unpubl.) in which similarly cannulated pigs were fed the same diet (Table IV.1) and infused with 800 ml water twice daily. As in a previous study (Just et al. 1981) no effects on these parameters were observed.

Intake of N by the 4 pigs during each period was not different ($P>0.05$) and averaged 160.4 g for both experiments (Tables IV.2 and 3). The N infused through the cannula (from CS, PC, SP or WB) was considered as N intake for calculation of N balance, N digestibility, biological value and N disappearance.

The AD of N from a SP-based diet (Table IV.1) and CS at the end of the small intestine of 4 cannulated pigs (56 to 60 kg body weight) were 80.1 (R. Misir and W.C. Sauer, Unpubl.) and 98.2% (Sauer et al. 1977a), respectively. In the current experiments, therefore, approximately 6.4 and 85.7 g/d of undigested N and starch, respectively, were expected to enter the hindgut. Additional N and starch were provided by infusion. The infusates and the ratio of energy

Table IV.2. Effect of infusion of cornstarch, pectin or wheat bran at the terminal ileum on nitrogen balance, urinary urea nitrogen and apparent biological value in pigs (exp. 1).

Parameter	Infusion[1]				SEM[2]
	Water	Cornstarch	Pectin	Wheat bran	
N intake, g/5 d	158.28	159.10	162.76	161.60	1.80
N excretion					
Fecal					
g/5 d	7.90[c][3]	12.12[b]	12.01[b]	17.44[a]	0.65
% N excretion[4]	9.07[b]	13.34[a]	11.79[a]	14.34[a]	0.71
Urinary					
g/5 d	80.10[b]	78.45[b]	90.23[b]	104.59[a]	2.73
% N excretion[4]	90.93[a]	86.66[b]	88.66[ab]	85.66[b]	0.70
N retained					
g/5 d	70.28	69.32	61.72	60.02	3.13
% N intake	44.40[a]	43.35[a]	37.64[ab]	32.98[b]	1.69
Urinary urea N, g/5 d	51.30[b]	49.38[b]	59.46[a]	61.52[a]	1.99
Apparent biological value, %	46.74[a]	46.90[a]	40.62[ab]	36.50[b]	1.77

[1]The substances and mean grams of N infused during the test periods were: cornstarch, 0.80; pectin, 1.20; and wheat bran, 20.45.

[2]Standard error of the mean.

[3]Means within a given row not followed by a common superscript are significantly different at $P<0.05$.

[4]Fecal + urinary N.

Table IV.3. Effect of infusion of cornstarch, soyprotein or cornstarch plus soyprotein at the terminal ileum on nitrogen balance, urinary urea nitrogen and apparent biological value in pigs (exp. 2).

	Infusion[1]				
Parameter	Water	Cornstarch	Soyprotein	Cornstarch + Soyprotein	SEM[2]
N intake, g/5 d	163.69	159.90	160.61	157.55	2.80
N excretion					
Fecal					
g/5 d	11.21[b][3]	16.44[a]	12.96[b]	18.16[a]	0.83
% N excretion[4]	10.91[b]	15.75[a]	10.23[b]	15.27[a]	0.74
Urinary					
g/5 d	91.90[c]	87.86[c]	114.81[a]	100.79[b]	2.41
% N excretion[4]	89.09[a]	84.25[b]	89.77[a]	84.73[b]	0.74
N retained					
g/5 d	60.57	56.40	64.73	71.30	3.65
% N intake	36.99	35.06	33.62	37.45	1.59
Urinary urea N, g/5 d	60.32[b]	59.12[b]	74.93[a]	67.95[ab]	2.33
Apparent biological value, %	39.72	39.05	36.07	41.41	1.53

[1]The substances and mean grams of N infused during the test periods were: cornstarch, 0.8; soyprotein, 31.9; cornstarch and soyprotein, 32.7.

[2]Standard error of the mean.

[3]Means within a given row not followed by a common superscript are significantly different at $P<0.05$.

[4]Fecal + urinary N.

to N (KJ/g) following infusions into the hindgut were: water (200), CS (667), PC (420) and WB (410) in experiment 1; or water (200), CS (660), SP (100) and CS plus SP (340) in experiment 2. SP was considered as a sole N source. Gargallo and Zimmerman (1981) had shown that the hindgut of the 40 kg pig could digest up to 150 g starch/d. In the present study therefore, the hindgut microbes were provided with adequate energy substrates (undigested and infused) in all pigs except those infused with water, PC or SP.

Fecal N consists mainly of microbial protein, the amount depending on the energy/N ratio in the hindgut (Mason and Palmer 1973, Mason et al. 1976, Mendez-Pereira et al. 1977). Most of the protein in the feces of pigs fed a corn-soybean meal diet was of microbial origin (Gargallo and Zimmerman 1981). In the present study microbial activity, as shown by greater fecal N output (g), increased ($P<0.05$) in response to the higher energy densities following infusion of CS (4.22), PC (4.11) and WB (9.54) in experiment 1; or CS (5.23) and CS plus SP (6.95) in experiment 2. The greater amount of fecal N (g) excreted in response to the infusion of WB (17.44), in contrast to CS (12.12) or PC (12.01), would suggest that the undigested and unabsorbed N from WB was making a greater contribution than the highly digestible SP to total fecal N (Table IV.2 and 3) Alternatively, both energy and N might be limiting for hindgut microbial activity when the pigs were fed the highly digestible SP diet and infused with water. The observation of similar

output of fecal N in both the pigs infused with CS or PC (which provided 3.06 or 1.53 MJ gross energy/d, respectively) indicated that the infused plus undigested dietary starch (285.7 g/d) was greatly in excess of the capacity of the hindgut for starch digestion (Gargallo and Zimmerman 1981).

Output of UN (g) was not affected ($P>0.05$) by the infusion of CS (Tables IV.2 and 3) or PC (Table IV.2), but was significantly increased ($P<0.05$) by infusions of WB (24.49), SP (22.91) and CS plus SP (8.89). As a percentage of the total N excretion, however, UN was only slightly decreased ($P<0.05$) by an elevation of the energy density in both experiments. Previous studies have shown greater decreases in total UN excretion following starch infusion in pigs fed protein sources of lower biological value such as meat-and-bone meal (Misir and Sauer 1980, Misir and Sauer 1981a), or barley and wheat (Misir and Sauer 1981b). In the current experiments, N might be limiting in the hindgut of the pigs infused with water, or the energy substrates (CS or PC).

The urinary urea N (g) was significantly increased ($P<0.05$) by infusion of PC (8.16), WB (10.22), SP (14.61), and CS plus SP (7.63). As a percentage of the infused N, increases were equivalent to 50.0, 45.8 and 23.3%, following infusions of WB, SP and CS plus SP, respectively (Tables IV.2 and 3). Since the infused PC provided only 1.2 g N as compared to WB (20.45 g) or CS plus SP (32.70 g), PC seemed

to exert this ureogenic effect by enhancing the catabolism and turnover rate of absorbed N, as was also observed for cholesterol metabolism in rats (Kelley and Tsai 1978). At lower energy densities (e.g., infusion of WB or SP, in contrast to CS or CS plus SP, respectively), more of the N was converted to ammonia (Fauconneau and Michel 1970, Grimson et al. 1971, Hodgdon 1977, Hodgdon et al. 1977, Deguchi et al.1979), absorbed into the hepatic portal blood (Hodgdon 1977, Hodgdon et al. 1977) and excreted in the urine largely as urea (Misir and Sauer 1981a,b), Tables IV.2 and 3. At higher energy densities, the hindgut microbes utilized the ammonia produced from the degraded protein for de novo synthesis of protein voided in the feces (Mason and Palmer 1973, Mason et al. 1976, Mendez-Pereira et al. 1977, Gargallo and Zimmerman 1981). Furthermore, the microbes utilized endogenous N substrates including blood urea (Deguchi et al. 1979, Bergner 1981, Mosenthin 1981), and amino acids (Holmes et al. 1974, Sauer et al. 1977b, Taverner et al. 1981). The utilization of endogenous N was also evident in this study (Table IV.4). High blood urea levels may also result when the animal is fed dietary protein levels greatly in excess of the NRC (1979) recommendations (P.A. Thacker and J.P. Bowland 1981, Pers. Comm.), or a poor quality protein such as meat-and-bone meal (Misir and Sauer 1980, 1981a).

Retention of N (g) was not affected ($P>0.05$) by the various treatments, and averaged 65.3 (experiment 1) or 63.3

(experiment 2); however, there was a noticeable though not significant ($P>0.05$) trend towards greater N retention in the pigs infused with SP (Table IV.3). The percentage N retained actually decreased ($P<0.05$) for the WB-infused pigs as compared to the water-infused pigs (i.e., 32.98 vs 44.40, Table IV.2). The amounts of N retained were paralleled by similar trends in measurements of apparent biological values (Tables IV.2 and 3). Increased N retention had previously been observed when casein was infused at the terminal ileum of pigs fed a protein-free diet (Zebrowska 1973) or a 16.1% protein, corn-soybean meal diet (Gargallo and Zimmerman 1981).

The total amount of nitrogenous residues (undigested and infused) available for fermentation in the hindgut, and the percentage of total N disappearance (i.e., net absorption) are presented in Table IV.4. Disappearance of N ranged from 49.61% (CS infusion) to 81.32% (SP infusion), and was influenced by infusions of both CS (average reduction, 13.98 percentage units for both experiments), and protein (SP, an increase of 6.59 percentage units). These data indicated the high capacity of the hindgut for digestion of N substrates (Zebrowska 1973, 1975, Gargallo and Zimmerman 1981, Just et al. 1981). The disappearance of the infused N (g), calculated by subtracting the increase in FN (g) due to CHO and/or protein infusion from the amount of N infused through the cannula (g) amounted to 10.91 (53.35%) for WB, or 30.15 (94.51%) for SP (Table IV.4). There seemed

Table IV.4. Total nitrogen entering the hindgut, nitrogen disappearance and apparent fecal digestibility of nitrogen by pigs infused with energy and protein sources at the terminal ileum.

	Total N[1]	N disappearance			Apparent fecal N dig.
		Total	Of infused N		
	g/5d	%	g/5d	%	%
Infusion (exp. 1)					
Water	32.57[b,2]	65.61[a]	0	-	94.97[a]
Cornstarch (CS)	32.62[b]	49.61[b]	-3.42	-	91.94[b]
Pectin	33.16[b]	60.98[a]	-2.91	-	91.94[b]
Wheat bran	51.80[a]	65.00[a]	10.91	53.35	79.17[c]
SEM[3]	0.56	1.65	-	-	0.44
Infusion (exp. 2)					
Water	31.50[b]	74.73[b]	0	-	93.16[a]
CS	32.46[b]	62.77[c]	-4.43	-	89.72[b]
Soyprotein (SP)	64.29[a]	81.32[a]	30.15	94.51	91.94[a]
CS + SP	64.86[a]	73.10[b]	25.75	78.75	88.50[b]
SEM	0.39	1.72	-	-	0.36

[1]Undigested + infused N.

[2]For each experiment, means within a given column not followed by the same superscript are significantly different at $P<0.05$.

[3]Standard error of the mean.

to be an interaction of energy and N on the metabolism of N in the hindgut since fewer grams of infused N apparently disappeared when the energy density was high, i.e., when CS plus SP as compared to SP alone was infused (i.e., 25.75 vs 30.15 g). When energy alone (i.e., CS, WB) was infused, the amounts of N disappearing in the hindgut exceeded that contributed by the ileal digesta plus infusate (Table IV.4). This observation clearly indicated that endogenous N was utilized by the microbes for de novo protein synthesis (Holmes et al. 1974, Bergner 1981, Mosenthin 1981).

All the pigs used in this study consumed equal amounts of the same diet (Table IV.1), yet the AD of the dietary N (percentage units) was significantly ($P<0.05$) lowered when CHO or CHO plus N were infused into the hindgut, i.e., CS, 3.03; PC, 3.03; WB, 15.80 in experiment 1; CS, 3.44; CS+SP, 4.66 in experiment 2. In normal animals (without cannulas), the amounts of energy substrates and protein residues entering the hindgut would depend on the ileal digestibilities of these dietary ingredients. The amounts of these residues, in turn, determine the extent of microbial fermentation, the amount of N excreted via the feces and the urine, and consequent estimates of apparent N digestibility (fecal analysis method, Kuiken and Lyman 1948), and apparent biological value measurements.

Classically, the amounts of N excreted via the feces and urine have been considered as completely discrete entities. The results of the present study have

substantiated previous data (Mason and Palmer 1973, Mason et al. 1976, Misir and Sauer 1980, 1981a,b) that the metabolism of N residues entering the hindgut represents the sum total of deamination and subsequent N incorporation by the microbes, thereby influencing the amount of N excreted by either pathway. Infused N was completely digested and absorbed but did not contribute to the protein status of the growing pig. The effect of the energy/N ratio on measurements of apparent N digestibility should be reasonable evidence for nutritionists to reexamine the currently held interpretation of amino acid availability, as determined by the fecal analysis method (Kuiken and Lyman 1948).

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V. COMPOSITION, AMINO ACID DIGESTIBILITIES AND PROTEIN QUALITY EVALUATION OF HIGH LYSINE BARLEYS[4]

A. ABSTRACT

Five experimental barley lines, bred and grown in Alberta under similar soil and environmental conditions, were evaluated as sole protein sources in two rat feeding trials. Chemical analyses showed 9 to 22% higher nitrogen (N) in Hiproly and the experimental lines (exp. 1) as compared to Galt barley (control). Amino acid (AA) analyses (exp. 1 and 2) also indicated higher lysine levels in Line 1 (29%), Hiproly (21%) and Line 4 (13%), and a reduction in the content of glutamic acid plus proline in Line 1 (16%) and Line 4 (6%), respectively. The data indicated that Line 1 could be classified as a "hiproly" barley, Line 4 as a "high lysine" barley, Line 2 a "high protein" barley, and Lines 3 and 5 "normal" barleys. In experiment 1, lysine was the least digestible AA but the values were similar ($P<0.05$) for all the barleys. In experiment 2, apparent digestibility of N in all the barleys was 10 -11 percentage units lower ($P<0.05$) than in casein. There was no difference ($P>0.05$) in performance of rats fed the barley lines and Galt. The relative protein values of the barleys reflected the lysine

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content of their respective protein. When the barleys are included as sole sources of both protein and energy in diets for 60 to 100 kg pigs, chemical score data showed that only Line 1 might adequately provide all the AA, including lysine. It is concluded that hiproly barleys are nutritionally superior, and would provide more grams of available lysine than normal barley.

B. INTRODUCTION

Cereal grains serve as major sources of nutrients, especially energy and protein, for monogastric animals. For optimal animal performance, the diets must provide adequate levels of all nutrients, including protein which supplies AA. However, the nutritional quality of cereal protein for monogastric animals is limited by the level of lysine, the first limiting AA (Munck 1976, Sauer 1976). In most cereals, the second limiting AA is threonine (Aw-Yong and Beames 1975, Sauer 1976). Current methods of diet preparation for rapidly growing animals involve mixing cereal grain, low in protein and indispensable AA, with more expensive protein supplements (e.g., soybean meal) which raise the protein content and improve the AA balance in the diet. Alternatively, the use of synthetic AA to rectify these deficiences has given satisfactory growth and feed conversion efficiencies when barley-based diets were fed to rats (Aw-Yong and Beames 1975, Beames 1977) and pigs (Braude et al. 1972, Chung and Beames 1974).

Supplementation of commercial barley- or wheat-based livestock diets with AA can be economically feasible only if adequate supplies are available at competitive prices (Braude et al. 1972, Aw-Yong and Beames 1974). However, lysine and methionine (including methionine hydroxy analogue) are the only synthetic AA produced as feed grade and the prices of these often reflect the price of expensive soybean meal (Beames 1977). Previous studies had shown high lysine barleys to be nutritionally superior in feeding trials with rats (Munck et al. 1970) and pigs (Thomke and Widstromer 1975). Accordingly, research efforts have turned to the development of cereal grains which contain indispensable AA, notably lysine, in adequate amounts and in the correct proportions to provide for optimum growth. The long-term objective of the barley breeding research in Alberta is to produce a barley which requires supplementation with only minerals and vitamins to serve as the sole diet for growing and finishing pigs. In the short term, however, the goal is to release for commercial production a barley cultivar which reduces the need for protein supplementation in the diets of all classes of pigs.

In "normal lysine" barleys, there is a negative correlation between the protein content of the grain and the lysine concentration in the protein; however, when expressed on a grain dry matter basis, the amounts of protein and lysine are positively correlated (Munck et al. 1971, Doll et al. 1974). "High lysine" barleys have higher lysine levels

than normal lysine barleys of similar protein contents (Munck et al. 1971, Doll et al. 1974), because lysine-rich albumins and globulins are increased at the expense of lysine-poor prolamines (Ingversen et al.1973, Balaravi et al. 1976). Alterations in the AA composition of the grain protein could be determined by chemical analyses, whereas improvement in nutritional quality of the protein is usually evaluated by animal feeding experiments.

Samples of new barley cultivars were available only in small amounts; consequently, the present study was conducted to (1) determine the crude protein and AA digestibilities of some barley lines, and (2) evaluate the protein quality in other barley lines, using the relative protein value (RPV) method (FAO/WHO 1975).

C. MATERIALS AND METHODS

Rat Management and Diet Preparation

Sprague-Dawley rats were assigned individually to metabolic cages (25 x 18 cm) with 1-cm mesh floors, which facilitated total collection of feces. The room was equipped with an automatic 12-h light-dark cycle (0700 to 1900 h) and maintained at a mean temperature of 24 ± 1°C.

The experimental barley lines used in the diets were developed at the Alberta Agricultural Research Station, Lacombe, grown under similar environmental and soil conditions, and harvested in 1977 (exp. 1) or 1978 (exp. 2). Prior to being mixed with the other dietary ingredients, all

grain samples were finely ground in a laboratory mill equipped with a 0.75 mm screen (Christy and Norris, Chelmsford, England). At the start of each experiment, the diet for each rat was weighed into a separate plastic cup with a tightly fitting lid. The daily dietary allowance for each rat was thereafter made from these cups and placed into food jars each equipped with a circular screen (1-cm pore size) and a metal cover (with a circular opening, 2.5 cm diameter) to minimize spillage. Water was provided ad libitum. During the test periods, feed not consumed plus feed spillage were determined; total feces produced by each rat were collected separately and pooled among days in the same plastic bag, frozen and stored at -20° C until required for analysis.

Experiment 1

Five-week old male rats, previously on a 12% crude protein commercial diet, were placed into weight groups from which 30 were randomly assigned into six treatment groups. The rats (mean initial weight ± SEM of rats on the different treatments = 90 ± 2 g) were put on a 9-d digestibility trial which consisted of a 4-d adaptation period followed by a 5-d test period (Eggum 1973). The experimental diets consisted of Galt (a normal commercial barley serving as the control), four test barleys, i.e., Hiproly (Munck et al. 1971), and three locally-developed lines. A Western Canadian utility wheat (Glenlea) was included in the experiment to compare the digestibilities of its AA with those of the

barleys. In all the diets (Table V.1) the grain was supplemented only with minerals and vitamins which met or exceeded the recommended levels (NRC 1978). A 15-g dietary allotment was given to each rat at 1100 h daily.

Experiment 2

Three-week old weanling rats were assigned on the basis of sex into weight groups from which they were randomly allocated to five treatment groups each consisting of 12 males and 12 females. The rats (mean initial weight ± SEM of both sexes = 55 ± 1 g) were fed ad libitum cornstarch-based diets formulated to contain 2, 5 or 8% crude protein (N x 6.25) from Galt, or one of two test barley lines. ANRC casein (Sheffield Chemical Co., Norwich, NY, USA) was used as the reference protein (Table V.2). The 14-d experimental period consisted of a 9-d adaptation period followed by a 5-d test period. Feed and water were removed from the cages 3 h before rats were reweighed at the end of the adaptation and test periods.

Calculations

Total feed intake and apparent digestibilities (AD) were determined as described (Chap. II). In experiment 1, the correction factors used in calculation of true digestibilities (TD) had been previously determined (Sauer 1976). In experiment 2, regression slope and correlation coefficients (body weight change on N intake) were calculated for casein and the test barley proteins (Snedecor and Cochran 1967). The relative protein value (RPV) of the

Table V.1. Composition and partial chemical analyses of the rat diets (exp. 1)

	Control barley	Test barleys[1]				Wheat
Diets:	Galt	Hiproly	Line 1	Line 2	Line 3	Glenlea
Ingredients (%, as fed)						
Grain	93.00	93.00	93.00	93.00	93.00	93.00
Calcium carbonate (38% Ca)	0.45	0.45	0.45	0.45	0.45	0.45
Calcium phosphate (17% Ca; 21% P)	1.90	1.90	1.90	1.90	1.90	1.90
Mineral mix[2]	2.40	2.40	2.40	2.40	2.40	2.40
Vitamin mix[3]	2.00	2.00	2.00	2.00	2.00	2.00
Chromic oxide	0.25	0.25	0.25	0.25	0.25	0.25
Chemical analyses (dry matter basis)[4]						
Crude protein, %	13.86±0.06	16.95±0.09	16.58±0.06	16.01±0.08	15.13±0.08	15.55±0.05
Crude fibre, %	3.02±0.01	1.03±0.01	2.37±0.04	3.11±0.02	2.69±0.02	1.84±0.01
Gross energy, MJ/kg	17.60±0.02	18.14±0.02	18.30±0.10	17.86±0.10	17.47±0.11	17.86±0.03

[1]Line 1 = H69024038000; Line 2 = H69019109000; Line 3 = H69024004191.

[2]Contributed the following nutrients per kilogram of diet: Na, 1.9 g; Cl, 2.9 g; Co, 0.16 mg; Cu, 10 mg; I, 0.23 mg, Fe, 65 mg; Mn, 50 mg; Se, 0.10 mg.

[3]Contributed the following vitamins per kilogram of diet: Vitamin A, 4,500 IU; vitamin D, 1,400 IU; alpha-tocopherol, 35 IU; menadione, 75 μg; choline, 1.25 g; folic acid, 25 mg; niacin, 30 mg; pantothenic acid, 10 mg; riboflavin, 5 mg; thiamin, 5; vitamin B_6, 8 mg; vitamin B_{12}, 50 μg.

[4]Average of two analyses ± standard error.

protein in a given test barley was the ratio of the regression slope coefficient, expressed as a fraction of that of casein (FAO/WHO 1975). Chemical score (CS) was calculated as the lysine content of the barley sample (g/16 g N), expressed as a percentage of the lysine level in the protein (g/100g) recommended for 60 to 100 kg pigs (NRC 1979).

Analyses

Proximate analyses were conducted on ground samples (0.75 mm) in duplicate. Grain, casein and freeze-dried feces for were analyzed for dry matter and Kjeldahl N; diets (exp. 1) for dry matter, Kjeldahl N, crude fibre and gross energy (AOAC 1970). Samples of grain, diets and feces (exp. 1), and casein and grain (exp. 2) were analyzed for AA as described by Sarwar and Bowland (1975). Analysis for tryptophan was according to the method of Hugli and Moore (1972).

Data (exp. 1) were subjected to one-way analysis of variance with significance among treatment means tested by the Student-Newman-Keuls' multiple range test (Snedecor and Cochran 1967).

D. RESULTS

Experiment 1

Chemical analyses (Table V.1) showed Hiproly diet to have the highest percentage crude protein (16.95) and Galt diet the lowest (13.86); the other diets (i.e., the barley lines and wheat) had intermediate values which were 1.27 to

2.72 percentage units higher than the control Galt diet. In addition, Hiproly diet had the lowest percentage of crude fibre (1.03), and Line 2 diet the highest (3.11).

Amino acid analyses of the grains (Table V.3) showed the lysine content (g/16 g N) to be highest in Line 1 (4.39), followed by Hiproly (4.10), Galt (3.39), Line 2 (3.34), Line 3 (3.00) and Glenlea wheat (2.36). As compared to the other barleys and wheat, Hiproly followed closely by Line 1, also had highest levels of threonine and methionine. Furthermore, Hiproly had the highest contents of the other indispensable AA. In general, the protein in the three barley lines contained similar levels of the other indispensable AA, which were the same as or higher than those in Galt or the wheat. Lines 1 and 2 also had lower levels (g/16 g N) of glutamic acid (20.39 and 22.06) and proline (10.10 and 11.03), respectively. All the barleys had similar contents of the other dispensable AA.

Digestibility Data

As compared to Galt, AD (percentage units) were lowest ($P<0.05$) in Line 2 for dry matter (80.6), in Hiproly for crude protein (73.4), and in Hiproly and Line 1 for valine (76.9 and 78.0), glutamic acid (86.4 and 86.0), proline (88.9 and 88.2), and serine (76.8 and 76.8), respectively (Table V.4). The true AA digestibilities for the barleys (Table V.5) showed basically the same pattern as the AD values (Table V.4) The values (percentage units) were lowest in Hiproly for crude protein (83.2), alanine (68.6) and

Table V.2. Composition of the rat diets (exp. 2)

Diets:	Casein			Control barley Galt			Test barley lines[1] Line 4			Line 5			Protein-free
Protein level, %[2]	2	5	8	2	5	8	2	5	8	2	5	8	0.4
Ingredients (% air dry basis)													
Barley	-	-	-	15.2	37.9	60.6	15.0	37.4	59.8	13.7	34.3	54.9	-
Casein	2.3	5.7	9.2	-	-	-	-	-	-	-	-	-	-
Cellulose[3]	1.0	1.9	3.0	-	-	-	-	-	-	-	-	-	1.0
Cornstarch	72.7	68.4	63.8	60.8	38.1	15.4	61.0	38.6	16.2	62.3	41.7	21.1	75.0
Common ingredients[4]	24.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0

[1]Line 4 = 69015134; Line 5 = H72033039.

[2]Calculated values based on Kjeldahl N x 6.25.

[3]Alpha floc, Lee Chemicals, 1119 Yonge Street, Toronto, Ontario.

[4]Dextrose, 10; soybean oil, 10; mineral mix, 3 (Bernhart and Tomarelli 1966) and vitamin mix 1 (Hegsted and Chang 1965).

Table V.3. Nitrogen content of the grains and partial amino acid analyses of the grain protein (exp. 1)

Samples[1]:	Control barley	Test barleys				Wheat
	Galt	Hiproly	Line 1	Line 2	Line 3	Glenlea
Nitrogen, % dry matter	2.39	2.92	2.85	2.75	2.60	2.68
Amino acids, g/16 g N						
Indispensable						
Arginine	4.84	5.02	4.76	4.32	4.42	4.24
Histidine	2.07	2.22	2.03	1.97	1.97	2.09
Isoleucine	3.32	3.68	3.38	3.38	3.32	3.16
Leucine	6.13	6.50	5.74	5.99	6.25	5.79
Lysine	3.39	4.10	4.39	3.34	3.00	2.36
Methionine	1.61	1.89	1.70	1.56	1.56	1.49
Phenylalanine	5.06	5.82	4.85	5.06	5.18	4.30
Threonine	3.63	4.09	3.88	3.65	3.60	3.26
Tryptophan	2.63	2.68	2.47	2.59	2.70	-
Valine	4.49	4.85	4.42	4.31	4.37	3.43
Dispensable						
Alanine	3.19	3.61	3.41	3.25	3.08	2.80
Aspartic acid	5.35	6.20	5.70	5.68	5.14	4.57
Glutamic acid	24.89	23.98	20.39	22.06	24.44	27.56
Glycine	3.36	3.63	3.55	3.34	3.32	3.58
Proline	11.38	12.17	10.10	11.03	12.03	9.06
Serine	4.07	4.18	3.70	4.03	3.90	4.29
Tyrosine	3.37	3.70	3.25	3.32	3.49	3.32

[1]Dry matter contents ± SEM (%) were Galt, 91.29 ± 0.06; Hiproly, 90.19 ± 0.06; Line 1, 91.98 ± 0.09; Line 2, 90.94 ±0.11; Line 3, 91.58 ± 0.01; Glenlea wheat, 89.33 ± 0.09.

Table V.4. Apparent digestibilities (%) of nitrogen and amino acids in barley and wheat diets (exp. 1)

Diets:	Control barley	Test barleys				Wheat	SEM[2]
	Galt	Hiproly	Line 1	Line 2	Line 3	Glenlea	
Dry matter	84.4^{bc}[1]	82.6^{c}	82.5^{c}	80.6^{cd}	85.0^{b}	88.5^{a}	0.6
Crude protein (N x 6.25)	77.9^{b}	73.4^{c}	77.1^{bc}	76.2^{bc}	79.4^{b}	86.9^{a}	1.1
Amino acids							
Indispensable							
Arginine	81.5^{b}	79.0^{b}	81.2^{b}	79.4^{b}	82.3^{b}	86.5^{a}	1.1
Histidine	89.6^{b}	86.8^{b}	88.3^{b}	88.1^{b}	89.0^{b}	93.6^{a}	1.1
Isoleucine	77.3^{b}	74.6^{b}	76.4^{b}	78.2^{b}	78.6^{b}	85.4^{a}	1.5
Leucine	80.3^{b}	77.1^{b}	77.8^{b}	80.3^{b}	82.0^{b}	87.4^{a}	1.2
Lysine	66.5^{a}	67.2^{a}	73.9^{a}	68.6^{a}	66.5^{a}	71.4^{a}	2.1
Methionine	71.9^{b}	71.1^{b}	73.3^{b}	75.8^{b}	74.3^{b}	82.7^{a}	1.6
Phenylalanine	84.7^{b}	83.7^{b}	82.3^{b}	84.1^{b}	86.1^{b}	89.6^{a}	0.9
Threonine	74.9^{b}	74.6^{b}	76.7^{b}	77.9^{b}	77.9^{b}	83.6^{a}	1.4
Valine	79.4^{ab}	76.9^{c}	78.0^{c}	79.6^{ab}	80.8^{ab}	83.7^{a}	1.3
Dispensable							
Alanine	65.7^{b}	62.7^{b}	66.6^{b}	70.5^{b}	66.9^{b}	77.7^{a}	2.0
Aspartic acid	68.0^{b}	67.6^{b}	69.5^{b}	74.3^{ab}	71.3^{ab}	78.7^{a}	2.2
Glutamic acid	89.9^{b}	86.4^{c}	86.0^{c}	89.6^{b}	90.4^{b}	95.5^{a}	0.7
Glycine	73.9^{b}	71.8^{b}	75.4^{b}	75.2^{b}	76.7^{b}	85.5^{a}	1.3
Proline	91.0^{b}	88.9^{c}	88.2^{c}	90.7^{b}	92.1^{b}	95.8^{a}	0.5
Serine	79.3^{bc}	76.8^{c}	76.8^{c}	82.7^{b}	81.3^{bc}	89.2^{a}	1.2
Tyrosine	78.9^{b}	77.1^{b}	78.4^{b}	79.8^{b}	81.6^{b}	86.9^{a}	1.2
Total	78.3^{b}	76.4^{b}	78.1^{b}	79.7^{b}	79.9^{b}	85.8^{a}	1.1

[1]Means in a given row followed by a common superscript are not significantly different at $P<0.05$.

[2]Standard error of the mean.

Table V.5. True digestibilities (%) of crude protein and amino acids in barley and wheat diets (exp. 1)

Diets:	Control barley	Test barleys				Wheat	SEM[2]
	Galt	Hiproly	Line 1	Line 2	Line 3	Glenlea	
Crude protein (N x 6.25)	90.0[a][1]	83.2[b]	86.0[ab]	87.3[a]	90.2[a]	-	1.1
Amino acids							
Indispensable							
Arginine	84.4[b]	81.2[b]	83.6[b]	82.2[b]	85.3[b]	89.5[a]	1.1
Histidine	92.4[b]	88.9[b]	90.7[b]	90.7[b]	91.8[b]	96.1[a]	1.1
Isoleucine	82.6[b]	78.4[b]	80.7[b]	82.6[b]	83.5[b]	90.4[a]	1.5
Leucine	85.3[b]	81.8[bc]	82.2[bc]	84.5[b]	86.6[b]	92.1[a]	1.2
Lysine	73.6[a]	71.9[a]	78.4[a]	74.7[a]	74.0[a]	80.5[a]	2.2
Methionine	77.2[b]	74.7[b]	77.4[b]	80.5[b]	79.5[b]	87.8[a]	1.6
Phenylalanine	88.0[bc]	86.0[bc]	85.2[c]	86.9[bc]	89.1[b]	93.1[a]	0.9
Threonine	81.2[b]	79.1[b]	81.6[b]	83.2[b]	83.9[b]	89.9[a]	1.4
Valine	84.3[b]	80.6[b]	82.2[b]	84.0[b]	85.5[ab]	89.4[a]	1.3
Dispensable							
Alanine	74.0[bc]	68.6[c]	73.0[bc]	77.5[b]	74.9[bc]	86.1[a]	2.1
Aspartic acid	76.0[b]	73.1[b]	75.8[b]	80.7[ab]	79.1[b]	87.1[a]	2.2
Glutamic acid	91.9[b]	88.1[c]	88.0[c]	91.6[b]	92.3[b]	97.2[a]	0.7
Glycine	80.9[bc]	77.0[c]	80.9[bc]	81.2[bc]	83.3[b]	91.4[a]	1.3
Proline	92.7[b]	90.2[c]	89.8[c]	92.2[b]	93.6[b]	97.7[a]	0.5
Serine	84.6[bc]	80.9[c]	81.6[c]	87.2[b]	86.5[b]	93.7[a]	1.2
Tyrosine	83.3[bc]	80.3[c]	82.2[bc]	83.6[bc]	85.6[b]	90.9[a]	1.2
Total	83.3[b]	80.0[b]	82.1[b]	84.0[b]	84.7[b]	90.8[a]	1.1

[1]Means in a given row followed by a common superscript are not significantly different at $P<0.05$.

[2]Standard error of the mean.

glycine (77.0); in Line 1 for phenylalanine (85.2); and in Hiproly and Line 1 for glutamic acid (88.1 and 88.0), proline (90.2 and 89.8) and serine (80.9 and 81.6), respectively. There was no difference (P>0.05) in the digestibility of lysine in the barley or wheat diets whether AD (Table V.4) or TD (Table V.5) was considered. In general, digestibilities of the other AA were lower for the barleys than for the wheat.

Experiment 2

The percentage N was basically the same for Line 4 (2.43), Line 5 (2.37) and Galt (2.40); however, AA analyses (Table V.6) showed higher lysine levels (g/16 g N) in Line 4 (3.75) and Line 5 (3.58) than Galt (3.33). Also, the levels of threonine and methionine plus cysteine were similar in these barley lines but slightly higher than those in Galt. Similar levels of the other indispensable AA were present in all the barleys. Among the dispensable AA, glutamic acid and proline levels were lowest in Line 4; the levels of the others were similar.

Protein Quality Data

The three dietary N levels, N intake and resulting changes in body weight, calculated values (regression slope coefficients, RPV's, correlation coefficients, and AD — for the 1.28% N diets only) are presented in Table V.7. The regression slope coefficient was highest (P<0.05) for casein (27.43), but was not significantly different (P>0.05) among the barleys, i.e., Galt (20.88), Line 4 (21.04), and Line 5

Table V.6. Nitrogen content and partial amino acid analyses of casein and barley samples (exp. 2)

Samples:	Casein	Control barley	Test barley lines	
		Galt	Line 4	Line 5
Nitrogen, % dry matter	15.22	2.40	2.43	2.37
Amino acid, g/16 g N				
Indispensable				
Arginine	3.82	4.73	4.80	4.66
Histidine	3.26	2.20	2.24	2.16
Isoleucine	5.33	3.46	3.48	3.51
Leucine	10.12	6.99	7.03	7.09
Lysine	8.64	3.33	3.75	3.58
Methionine	3.20	1.26	1.38	1.42
Phenylalanine	5.45	5.39	5.33	5.54
Threonine	4.23	3.26	3.48	3.45
Valine	6.83	5.13	5.00	5.00
Dispensable				
Alanine	3.16	3.93	4.08	3.92
Aspartic acid	7.51	5.59	6.31	6.01
Cysteine	0.38	2.14	2.24	2.16
Glutamic acid	23.57	26.90	25.58	26.76
Glycine	1.95	3.79	4.08	3.92
Proline	11.22	11.65	10.85	11.28
Serine	6.21	4.33	4.34	4.26
Tyrosine	5.99	2.06	2.04	2.09

Table V.7. Rat growth performance, regression slope and correlation coefficients, relative protein value (RPV) and apparent digestibility (AD) of casein and barley protein (exp. 2)

Protein source	N content of diets (%)	N intake (g/14 d)[1]	Body weight gain (g/14 d)[1]	Regression coefficient of slope	RPV	Correlation coefficient r	AD %[1]
Casein	0.32	0.305 ± 0.035	-3.0 ± 2.14				
	0.80	1.260 ± 0.154	21.8 ± 6.52				
	1.28	2.190 ± 0.239	48.0 ± 9.36				
				27.43 ± 3.28[a2]	1.000	0.989**	89.9 ± 1.82[A2]
Normal barley							
Galt	0.32	0.340 ± 0.046	-3.9 ± 2.17				
	0.80	1.110 ± 0.073	11.7 ± 2.18				
	1.28	2.130 ± 0.257	32.4 ± 9.77				
				20.88 ± 3.21[b]	0.761	0.968**	78.8 ± 1.81[B]
Test barley lines							
Line 4[1]	0.32	0.340 ± 0.022	-3.9 ± 0.81				
	0.80	1.170 ± 0.068	13.7 ± 3.30				
	1.28	2.120 ± 0.287	32.2 ±10.62				
				21.04 ± 3.67[b]	0.767	0.967**	79.4 ± 1.20[B]
Line 5	0.32	0.320 ± 0.055	-3.8 ± 2.14				
	0.80	1.160 ± 0.180	13.5 ± 3.27				
	1.28	2.160 ± 0.195	33.4 ± 8.39				
				20.56 ± 3.48[b]	0.750	0.968**	79.8 ± 1.36[B]

1 Mean ± standard deviation.
2 Means in a given column not followed by the same superscript are significantly different: a, b at $P < 0.05$; A, B at $P < 0.01$.
** $P < 0.01$

(20.56); this was reflected in similar RPV's for the barleys. The correlation coefficients reached very high levels and were significant ($P<0.01$) all the test proteins, including casein (0.989), Galt (0.968), Line 4 (0.967) and Line 5 (0.968). All the barleys showed similar apparent protein digestibilities which were 10.1-11.1 percentage units lower ($P<0.01$) than the value for casein (89.9%).

E. DISCUSSION

Feed costs may account for 50-70%, or even more, of total production cost in intensive livestock operations. In Alberta and in other areas of Western Canada, barley is the cereal grain most commonly used in animal diets. Since barley makes a larger single contribution of both energy and protein than any other ingredient in pig diets, any increase in the lysine content and consequent improvement in protein quality would reduce the need for supplementation with protein ingredients. This would result in substantially lower feed costs and therefore greater profit to the producer. More of the conventional protein sources, e.g., soybean meal, could then be diverted for direct human consumption.

Crude protein and lysine levels of the Hiproly sample used in this study fell within the range reported (Munck et al. 1971). In experiment 1, the lysine levels in Line 1 and Hiproly were markedly higher than that of Galt by 29 and 21%, respectively. In addition, Line 1 showed 16% less

glutamic acid plus proline. Similar but smaller changes were observed in Line 4 (exp. 2) for lysine (13% increase) and glutamic acid plus proline (6% decrease). The increases in lysine content along with the simultaneous decrease in glutamic acid plus proline concentrations would suggest alteration in the distribution of the constituent grain protein fractions. Previous workers (e.g., Balaravi et al. 1976, Munck 1976) had reported increases in lysine-rich albumins and globulins, and concomitant decreases in lysine-poor prolamines. Among the barleys used in the current study, there was also an increase in the protein content in Hiproly (22%) and Line 1 (19%) but not in Line 4; therefore, Line 1 could be classified as a "high protein, high lysine" or "hiproly" barley, and Line 4 a "high lysine" barley. Furthermore, all these barleys had higher levels of threonine and methionine, thereby improving the balance of indispensable AA. Ingversen et al. (1973) and Helm (1977) had found increased levels of threonine in high lysine mutants. Beames (1977) obtained a positive correlation ($r=0.79$) between lysine and threonine contents in high lysine barley lines grown in Western Canada. Therefore, in selecting for the high-lysine trait, the plant breeder is also selecting for threonine, the second limiting AA (Aw-Yong and Beames 1975, Sauer 1976). As compared to Galt, Line 2 had similar lysine but higher protein content (15%); Lines 3 and 5 both had similar protein and lysine levels as the corresponding controls. Accordingly, Line 2 is a "high

protein" barley whereas Lines 3 and 5 are "normal" barleys (Munck et al. 1971, Doll et al. 1974).

As the sole source of protein for 60 to 100 kg pigs, all the barleys including controls, would provide adequate levels of all indispensable AA except lysine (NRC 1979). The chemical score (CS) for lysine indicated that only Line 1 (CS=100) might also be adequate in this AA. Hiproly (CS=94), Line 4 (CS=86) and Line 5 (CS=82) and Galt (CS=76, exp. 1; CS=77, exp. 2); Line 2 (CS=76), Line 3 (CS=68), as well as wheat (CS=54), would require supplementation with varying amounts of soybean meal.

The lysine-rich albumins and globulins, which have a relatively balanced AA composition (Pomeranz 1975, Rhodes and Gill 1980) are confined largely to the aleurone cells of the cereal grain embryo (Sauer 1976, Eggum 1977). The barley kernel has a thick-walled, multicellular aleurone layer; in contrast, wheat has only a single-celled aleurone layer (Postel 1956, Eggum 1977). Previous studies with rats (Eggum 1973) and pigs (Sauer et al. 1974, 1977, 1981; Misir and Sauer 1981b) had shown lower AA digestibilities in barley as compared to wheat. It is likely that when barley is fed, the thick cellulosic walls of a substantial percentage of the aleurone cells are not broken down in the gut, as shown in studies with chicks (Saunders et al. 1969), thereby reducing contact with proteolytic digestive enzymes. This would partly explain the lower digestibilities of AA in the barleys as compared to Glenlea wheat. The similar

digestibility of lysine observed for both barley and wheat might be explained by higher concentration of lysine in the aleurone cells relative to the endosperm.

As compared to normal barley controls, lower true digestibility of N (or crude protein) had been obtained in feeding high lysine barleys to rats. In one study (Johnson et al. 1978) the TD of N and lysine were reduced by 4.0 and 6.5 percentage units, respectively; however, the amount (g/16 g N) of lysine available for absorption actually increased by 17% because of the higher lysine content in the grain. Doll et al. (1974) found no evidence of reduction of the TD of N and lysine in rats. In contrast, higher TD of N and lysine had been obtained when high lysine barleys had been fed to rats (Munck et al. 1970) or pigs (Thomke and Widstromer 1975). In the current study (exp. 1), the amount of lysine available for absorption and subsequent protein synthesis (i.e., lysine intake,g x lysine digestibility,%) seemed to be directly related to the lysine content in the grain .

Protein quality, as measured by RPV's, was the same in Galt and in Lines 4 and 5, but lower ($P<0.05$) by 23-25% than that of casein. When used in practical feeding, these barleys would thus require supplementation with high protein ingredients to improve the overall quality of the dietary protein. The high correlation coefficients would suggest that the different barleys were utilized with equal efficiency at all levels of dietary intake.

A major problem which precludes the cultivation of high lysine barleys is the reported low yields (Munck et al. 1970; Helm 1977; Newman et al. 1978), resulting from shrunken kernels (Doll et al. 1974, Newman et al. 1978) and thus lower kernel weight (Doll et al. 1974, Bansal et al. 1977). Persson and Karlsson (1977), however, obtained yields in high lysine barleys comparable to those of normal high-yielding controls. If the yields in Hiproly, and Lines 1 and 4, used in the present study, could be improved by genetic manipulation then the resulting high yielding, high lysine barleys could be released for commercial production.

Various researchers, including Munck et al. (1970, 1971) and Newman et al. (1974) had found the nutritional value of high lysine barleys to be superior to that of normal control barley. Beames (1977) showed that growth rates and feed conversion efficiencies were similar when weanling rats were fed Galt barley plus synthetic lysine, or unsupplemented high lysine barley diets. However, rat growth performance was improved when soybean meal was added to the diets, indicating deficiencies of one or more AA in the high lysine barleys fed. Beames (1977) attributed the inadequacy of these high lysine barleys to the higher requirements for AA by the weanling rat relative to the growing pig (NRC 1979). In studies with growing–finishing pigs, Newman et al. (1978) reported considerable savings in soybean meal (as the protein supplement) when the cereal component in the diet was high lysine barley.

In studies with pigs fed barley-based diets, the fecal analysis method (Kuiken and Lyman 1948) gave similar AD estimates of lysine and methionine as those measured at the terminal ileum, but overestimated those of other indispensable AA, e.g., threonine and histidine in a barley diet by 10.2 and 11.5 percentage units, respectively (Sauer et al. 1977). For wheat diets, this method overestimated the AD of lysine, threonine and methionine by more than 8 percentage units in each case (Sauer et al. 1981). Of practical consideration are threonine, the second limiting AA in barleys (Aw-Yong and Beames 1975, Sauer 1976), and lysine and threonine, first and second limiting AA respectively, in wheat (Ivan and Farrel 1973). Therefore, care must be exercised in the interpretation of fecal digestibility values for all AA except lysine and methionine (barley-based diets) and in their subsequent application in diet formulation.

The results of the current study indicate that high lysine barleys offer promise in meeting the stated objectives of plant breeders and animal nutrition scientists. Chemical analyses suggest superior nutritional potential of Hiproly and the test barleys Lines 1 and 4. For pigs (60 to 100 kg), all the barleys had adequate levels of the indispensable AA except lysine. CS for lysine indicates that Line 1 might adequately meet the total AA requirements for this class of pigs. It is likely, however, that all the barleys including Line 1, may still require supplementation

with AA or protein (though at a reduced level) in the diet, since the NRC (1979) estimates are based on available AA from corn-soybean meal diets whereas the CS values in this study were calculated using the analyzed levels of AA in the barley and wheat samples. In general, the amount of lysine available to the pig is expected to be higher when hiproly or high lysine barleys as contrasted to high protein or normal barleys are fed. Clearly, feeding experiments with growing–finishing pigs must be conducted to evaluate more accurately the protein quality and adequacy of the barleys.

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VI. COMPOSITION AND BIOLOGICAL EVALUATION OF HIGH LYSINE BARLEYS BY GROWING RATS AND PIGS[5]

A. ABSTRACT

One established high lysine barley (Hiproly), one experimental high lysine barley mutant (Risø 1508) and one experimental barley line (Line 6), all grown in Alberta under similar soil and environmental conditions were evaluated as sole protein sources in one rat and one pig experiment. As compared to Galt (a normal control barley), crude protein contents were 67, 47 and 16% higher in Hiproly, Line 6 and Risø 1508, respectively. The lysine content (%) in the grain, and the percentage increase relative to Galt were Hiproly (0.72, 80%), Risø 1508 (0.71, 78%) and Line 6 (0.58, 45%), respectively. In the rat trial, relative protein values (RPV's) expressed as a percentage of that of Galt, were 115, 109 and 108 for Line 6, Hiproly and Risø 1508, respectively. In the pig trial, mean nitrogen (N) retention (as a percentage of intake N) was similar for test barleys (43.5) but higher ($P<0.01$) than for Galt (32.5). In both trials, the true digestibilities (TD) of N (crude protein) for all the barleys followed basically the same trend but were consistently higher than apparent

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digestibilities (AD); however, both the AD and TD values were lower for pigs than for rats. Apparent biological value (%) was lowest ($P<0.01$) for Galt (50.4), and highest for Risø 1508 (66.0) but not significantly different ($P>0.01$) from that of Hiproly (57.8) or Line 6 (58.7). The high biological value of Risø 1508, despite the low TD of N was directly related to amount (g) of lysine available for absorption by the pig. It is concluded that Alberta grown high lysine barleys are nutritionally superior to a normal control barley and should reduce the need for supplementary protein in diets for growing-finishing pigs.

B. INTRODUCTION

The search for cereal grains high in lysine led to the discovery of high lysine barleys (Munck et al. 1971), corn (Mertz et al. 1964, Nelson et al. 1965) and sorghum (Singh and Axtell 1973). Since the nutritional value of cereal proteins is limited by the level of lysine (Munck 1976, Sauer 1976, Eggum 1977), the use of high lysine cereals in livestock diets is expected to reduce protein supplementation, resulting in lower total feed costs.

Nutritional studies have shown that the protein quality of high lysine barleys is superior to that of normal barleys when fed to rats (Munck et al. 1970, Munck 1972, Doll et al. 1974, Balaravi et al. 1976, Stobart 1977), and pigs (Thomke and Widstromer 1975). In practical pig diets, the use of high lysine barleys resulted in substantial savings of

soybean meal (Thomke and Widstromer 1975, Newman et al. 1978). Amino acid analyses indicated high lysine barley to be potentially capable of meeting the total amino acid requirements (NRC 1979) for 60 to 100 kg pigs (Chap. V).

Preliminary information on the protein quality of experimental barley lines is obtained by chemical evaluation (Munck et al. 1971, Ingversen and Koie 1973, Rhodes and Gill 1980), and both chemical and biological evaluation using rats (Doll et al. 1974, Newman et al. 1974, Balaravi et al. 1976, Bansal et al. 1977, Stobart 1977, Misir and Sauer 1981c). It is important to have such information available in order to identify experimental lines that might be nutritionally superior when included in diets for domestic livestock species. Therefore, the objective of the present study was to evaluate, by chemical analyses and in feeding trials using both growing rats and pigs, the protein quality of Alberta grown barleys, i.e., Hiproly, an established high protein, high lysine barley (Munck et al. 1971), Risø 1508, a high lysine barley mutant (Ingversen et al. 1973) and Line 6 (a locally bred barley line).

C. MATERIALS AND METHODS

All the barleys were grown in Alberta in 1978 under similar environmental and soil conditions. Line 6 was developed at the Alberta Agricultural Research Station, Lacombe. The barleys used in both experiments were taken from the same batch.

Experiment 1

Three-week old weanling rats were assigned on the basis of sex into weight groups from which they were randomly allocated to five treatment groups each consisting of 12 males and 12 females. The mean initial weight ± SEM for rats of both sexes was 52 ± 1 g. Nitrogen (N) content and partial amino acid analyses of the barleys are presented in Table VI.1. The rats were fed ad libitum cornstarch-based diets, formulated to contain 2, 5 and 8% crude protein (N x 6.25) from casein, and each of the barleys: Galt, Hiproly, Risø 1508, and Line 6. ANRC casein (Sheffield Chemical Co., Norwich, NY, USA) was used as the reference protein. Rat management, room conditions, diet preparation and composition, procedures for collection and storage of samples prior to chemical analyses, were previously described (Chap. V). The rats were put on a 14-d experimental period which consisted of a 9-d adaptation period followed by a 5-d period for total collection of feces. Immediately thereafter, the rats were fed a protein-free diet (Table V.2) for a similar experimental period. Feed and water were removed from the cages 3 h before the rats were reweighed at the end of both adaptation and collection periods.

Experiment 2

Eight Yorkshire x Lacombe barrows, ranging in initial liveweight from 43 to 47 kg, were individually confined to metabolic cages that permitted separate collection of feces

Table VI.1. Nitrogen content and partial amino acid analyses of the protein of the barley samples (exp. 1 and 2).

Barleys	Galt	Hiproly	Risø 1508	Line 6[1]
Nitrogen, % dry matter	1.72	2.88	2.00	2.53
Amino acids, g/16 g N				
<u>Indispensable</u>				
Arginine	4.91 (0.53)[2]	4.59 (0.83)	6.73 (0.84)	4.67 (0.74)
Histidine	2.17 (0.23)	2.13 (0.38)	2.94 (0.37)	2.04 (0.32)
Isoleucine	3.48 (0.37)	3.65 (0.66)	3.67 (0.46)	3.53 (0.56)
Leucine	6.98 (0.75)	7.01 (1.26)	7.32 (0.91)	6.82 (1.08)
Lysine	3.74 (0.40)	4.01 (0.72)	5.71 (0.71)	3.67 (0.58)
Methionine	1.22 (0.13)	1.37 (0.25)	1.51 (0.19)	1.21 (0.19)
Phenylalanine	4.74 (0.51)	5.72 (1.03)	4.22 (0.53)	5.01 (0.79)
Threonine	3.29 (0.35)	3.34 (0.60)	4.32 (0.54)	3.27 (0.52)
Valine	4.22 (0.45)	4.19 (0.75)	4.93 (0.62)	4.11 (0.65)
<u>Dispensable</u>				
Alanine	3.87 (0.42)	4.13 (0.74)	5.35 (0.67)	3.86 (0.61)
Aspartic acid	6.25 (0.67)	6.50 (1.17)	9.54 (1.19)	6.14 (0.97)
Cysteine	1.96 (0.22)	1.09 (0.20)	1.96 (0.24)	1.51 (0.24)
Glutamic acid	22.13 (2.38)	23.36 (4.20)	17.48 (2.18)	23.29 (3.68)
Glycine	3.81 (0.41)	3.78 (0.68)	5.57 (0.69)	3.68 (0.58)
Proline	10.04 (1.08)	11.05 (1.99)	7.04 (0.88)	10.60 (1.67)
Serine	4.15 (0.45)	4.17 (0.75)	4.76 (0.59)	4.14 (0.65)
Tyrosine	2.23 (0.24)	2.19 (0.39)	2.42 (0.30)	2.07 (0.33)

[1]NS #2.

[2]Values in parentheses refer to amino acid content expressed as a percentage of the grain (dry matter basis).

and urine. Room conditions and procedures for collection and storage of samples have been described (Chap. II). Prior to being put on this experiment, the pigs had been fed a University of Alberta barley-soybean meal grower diet (16% crude protein).

The experimental diets (Table VI.2) were prepared using the barleys (Table VI.1) and were formulated to be isonitrogenous and isocaloric by the addition of alpha floc (Lee Chemicals, 1119 Yonge Street, Toronto, Ontario) and cornstarch, respectively. Minerals and vitamins were added to meet or exceed NRC (1979) specifications. The barleys were ground (3 mm particle size) before being mixed with the other ingredients. Nitrogen content and partial amino acid analyses of the diets are presented in Table VI.3.

Each experimental period lasted 10 d during which each pig was given 900 g diet twice daily at 0700 h and 1900 h. Water was supplied ad libitum. Daily collections of total feces and urine output were made during the last 5 d of each period.

Chemical analyses

Proximate analyses were conducted on ground samples (0.8 mm) in duplicate. Grain (exp. 1 and 2), casein, diets and composite freeze-dried feces (exp. 1), diets and oven-dried (60°C) feces (exp. 2) were analyzed for dry matter and Kjeldahl N (AOAC 1970). Amino acid analyses were conducted on grain samples, pig diets, and feces (pooled among days for each period) as described by Sarwar and

Table VI.2. Composition and partial chemical analyses of the pig diets (exp. 2).

Diets:	Galt	Hiproly	Risø	Line 6
Ingredients, % as fed				
Barley	94.8	57.4	82.5	64.7
Cornstarch	-	33.9	9.7	27.4
Alpha floc	-	3.0	2.5	2.4
Soybean oil	3.0	3.0	3.0	3.0
Calcium carbonate (38% Ca)	1.2	0.9	1.1	0.9
Calcium phosphate (17% Ca, 21% P)	0.5	1.3	0.7	1.1
Mineral mix[1]	0.3	0.3	0.3	0.3
Vitamin mix[2]	0.2	0.2	0.2	0.2
Chemical analyses, % as fed[3]				
Crude protein	9.12±0.01	9.26±0.03	9.24±0.02	9.15±0.04
Dry matter	89.44±0.32	89.63±0.21	89.77±0.19	89.60±0.23

[1]Contributed the following nutrients per kilogram of diet: Na, 1.9 g; Cl, 2.9 g; Co, 0.16 mg; Cu, 10 mg; I, 0.23 mg; Fe, 65 mg; Mn, 50 mg; Se, 0.10 mg.

[2]Contributed the following vitamins per kilogram of diet: Vitamin A, 4,500 IU; vitamin D, 1,400 IU; alpha-tocopherol, 35 IU; menadione, 75 μg; choline, 1.25 g; folic acid, 25 mg; niacin, 30 mg; pantothenic acid, 10 mg; riboflavin, 5 mg; thiamin, 5; vitamin B_6, 8 mg; vitamin B_{12}, 50 μg.

[3]Average of two analyses ± standard error.

Table VI.3. Nitrogen content and partial amino acid analyses of the pig diets (exp. 2).

Barleys	Galt	Hiproly	Risø 1508	Line 6
Nitrogen, % dry matter	1.63	1.65	1.65	1.63
Amino acids (%, as fed)				
Indispensable				
Arginine	0.45	0.42	0.62	0.43
Histidine	0.20	0.20	0.27	0.19
Isoleucine	0.32	0.34	0.34	0.32
Leucine	0.64	0.65	0.68	0.62
Lysine	0.34	0.37	0.53	0.34
Methionine	0.11	0.13	0.14	0.11
Phenylalanine	0.43	0.53	0.39	0.46
Threonine	0.30	0.31	0.40	0.30
Valine	0.39	0.39	0.46	0.38
Dispensable				
Alanine	0.35	0.38	0.50	0.35
Aspartic acid	0.57	0.60	0.88	0.56
Cysteine	0.18	0.10	0.18	0.14
Glutamic acid	2.02	2.16	1.62	2.13
Glycine	0.35	0.35	0.52	0.34
Proline	0.92	1.02	0.65	0.97
Serine	0.38	0.39	0.44	0.38
Tyrosine	0.20	0.20	0.22	0.19

Bowland 1975).

Calculations and Statistical Analysis

Chemical scores were calculated as described (Chap. V). Apparent digestibility (AD) of a given nutrient was considered as the difference between the total amounts (g) consumed and voided in the feces, expressed as a percentage of the total (g) nutrient consumed (Kuiken and Lyman 1948). True digestibilities (TD) were calculated using the metabolic fecal N values (Table VI.4, footnote 2) obtained when the rats (exp. 1) were fed the protein free diet (Table V.2), or other correction factors for the pigs (exp. 2), previously determined (H. Jørgensen and W.C Sauer 1981, Pers. Comm.). In experiment 1, regression slope and correlation coefficients (body weight changes on N consumed) were calculated for casein and the test barley proteins (Snedecor and Cochran 1967). RPV's were determined using the regression slope coefficients of the test protein, expressed as a fraction of that of casein (FAO/WHO 1975). In experiment 2, apparent biological value was taken as the percentage of the apparently absorbed N that was retained.

The data for the four barleys were subjected to one-way analysis of variance (exp. 1), or analyzed using a double (concurrent) 4x4 Latin Square experiment of barleys x barrows (exp. 2). In both experiments, significance among treatment means were tested by the Student-Newman-Keuls' multiple range test (Snedecor and Cochran 1967).

Table VI.4. Regression slope and correlation coefficients, relative protein values (RPV) and nitrogen digestibilities of casein and barley protein (exp. 1).

Protein source	Regression coefficient of slope	Correlation Coefficient	RPV	N Digestibility[1] Apparent	True[2]
	b_1	r		%	%
Casein	27.43 ± 0.95^{a}[3]	0.986**	1.000	89.9 ± 1.78^{A}[3]	95.2 ± 1.02^{A}
Galt	$19.17 + 0.98^{b}$	0.964**	0.699	76.3 ± 1.83^{B}	81.3 ± 2.04^{B}
Hiproly	20.96 ± 0.72^{b}	0.972**	0.764	78.4 ± 1.21^{B}	83.4 ± 1.32^{B}
Risø 1508	20.74 ± 0.62^{b}	0.962**	0.756	73.3 ± 1.42^{BC}	78.4 ± 1.49^{BC}
Line 6	22.14 ± 0.83^{b}	0.975**	0.807	75.7 ± 1.39^{B}	81.1 ± 1.45^{B}

[1]Values were calculated for the 8% crude protein level only. Mean ± standard deviation.

[2]Metabolic fecal N was determined as 9.3 mg/100 g body weight/d.

[3]Means in a given column followed by a common superscript are significantly different: A,B,C at $P<0.01$; a,b at $P<0.05$.

D. RESULTS

Chemical Analyses

Chemical analyses of the barleys used in both the rat and the pig experiments (Table VI.1) showed Hiproly to have the highest percentage N (2.88), and Galt (control) the lowest (1.72); Line 6 and Risø 1508 had intermediate values, i.e., 2.53 and 2.00, respectively. The levels of lysine (g/16 g N) were higher than those of threonine but followed the same trend in all the barleys, i.e., Risø 1508 (5.71 and 4.32), Hiproly (4.01 and 3.34), Galt (3.74 and 3.29) and Line 6 (3.67 and 3.27), respectively. In addition, the level of methionine plus cysteine was highest for Risø 1508 (3.47), followed by Galt (3.18), Line 6 (2.72) and Hiproly (2.46). Among the barleys, Risø 1508 had the lowest levels of glutamic acid (17.48) and proline (7.04).

Experiment 1

The regression slope and correlation coefficients, RPV's, AD and TD of N in casein and the barleys are presented in Table VI.4. The value for the regression slope coefficient was highest for casein (27.43) as compared to those for the barley proteins, but were significantly lower ($P<0.05$), i.e., Galt (19.17), Hiproly (20.96), Risø 1508 (20.74) and Line 6 (22.14). Differences among regression slope coefficients were reflected in their RPV's, i.e., 1.000, 0.807, 0.764, 0.756 and 0.699 for casein, Line 6, Hiproly, Risø 1508 and Galt, respectively. Digestibility values were higher for casein ($P<0.01$) than those of the

barleys. The TD values for N (percentage units) were 95.2 (casein), 83.4 (Hiproly), 81.3 (Galt), 81.1 (Line 6), and 78.4 Risø 1508; these values were 5.0 – 5.4 percentage units higher than corresponding AD values.

Experiment 2

Nitrogen Balance Data

The mean N intake (129.0 g) of the pigs fed the 4 barley diets was not different (P>0.01); however, the performance of pigs varied for the response criteria measured (Table VI.5). As a percentage of intake N, pigs fed the Hiproly or Line 6 diets excreted similar but lower levels of N (P<0.05) in the feces (25.3 or 27.8, respectively) as compared to those pigs fed Galt (35.4) or Risø 1508 (32.2). Excretion of urinary N was less (P<0.05) by the pigs fed Risø 1508 (23.1) relative to Galt (32.0), Hiproly (31.5) or Line 6 (29.6). Similar levels of N were retained by the pigs fed Hiproly (43.2), Risø 1508 (44.7) or Line 6 (42.6); these values were higher (P<0.01) than that for the pigs fed Galt (32.5). Apparent biological values (%) were higher (P<0.01) than corresponding N retention values but followed the same basic pattern, i.e., 57.8 (Hiproly), 66.0 (Risø 1508), 58.7 (Line 6) and 50.4 (Galt).

Digestibility Data

For the three test barleys, both AD and TD of N, indispensable AA and dispensable AA except cysteine, were similar or higher than corresponding values for Galt (Tables VI.6 and VI.7). The AD of crude protein was highest (P<0.01)

Table VI.5. Nitrogen balance and apparent biological value of high lysine barley diets (exp. 2).

Diet:	Galt	Hiproly	Risø 1508	Line 6	SEM[1]
Intake N, g/5d	127.9	130.2	129.6	128.4	0.2
Fecal N, % intake N	35.4^{a}[2]	25.3^{b}	32.2^{a}	27.8^{b}	1.3
Urinary N, % intake N	32.0^{a}	31.5^{a}	23.1^{b}	29.6^{a}	1.7
Retained N, % intake N	32.5^{B}[2]	43.2^{A}	44.7^{A}	42.6^{A}	2.1
Apparent biological value, %	50.4^{B}	57.8^{AB}	66.0^{A}	58.7^{AB}	2.4

[1]Standard error of the mean.

[2]Means within a given row followed by a common superscript are not significantly different: A, B, C at $P<0.01$; a, b at $P<0.05$.

in Hiproly (74.7), lowest in Galt (64.6), and intermediate in Line 6 (72.2) and Risø 1508 (67.8). Among the indispensable AA, the AD (%) of lysine and threonine, respectively, were higher ($P<0.01$) in Hiproly (67.1, 70.1), Risø 1508 (67.2, 68.2) or Line 6 (62.2, 68.6) than in Galt (50.6, 62.0). The AD (%) of methionine was not different in Hiproly (61.7) or Risø 1508 (57.5), but was higher ($P<0.05$) than in Line 6 (53.1) or Galt (53.6). TD of N, indispensable and dispensable AA followed the same trend, but were higher than corresponding AD values.

E. DISCUSSION

Chemical Evaluation

Previous chemical evaluation studies (Munck et al. 1971, Ingversen et al. 1973) indicated marked increases in the levels of lysine in high lysine barleys. In the present study, the increased lysine levels of the barleys (as a percentage relative to Galt) were 80, 78 and 45 for Hiproly, Risø 1508 and Line 6, respectively (Table VI.1). The level of threonine, the second limiting amino acid in barleys (Ingversen et al. 1973, Aw-Yong and Beames 1975, Sauer 1976) showed a 31% increase (g/16g N) in Risø 1508. A previous study in Denmark, which compared Risø 1508 to its parent Bomi (Ingversen et al. 1973), reported similar increases for lysine and threonine, plus marked increases for arginine, histidine, aspartic acid, glycine and alanine, in addition to decreases in glutamic acid and proline. Clearly, the

Table VI.6. Apparent digestibilities (%) of dry matter, crude protein and amino acids in the barley diets by pigs (exp. 2).

Barleys	Galt	Hiproly	Risø	Line 6	SEM[1]
Dry matter	80.0^{B}[2]	87.7^{A}	78.1^{B}	85.6^{A}	0.9
Crude protein	64.6^{C}	74.7^{A}	67.8^{BC}	72.2^{AB}	1.3
Amino acids					
Indispensable					
Arginine	78.3^{b}[2]	82.0^{ab}	84.0^{a}	82.6^{ab}	0.7
Histidine	81.0^{a}	83.0^{a}	84.6^{a}	82.9^{a}	0.6
Isoleucine	61.6^{B}	73.1^{A}	64.8^{B}	70.7^{A}	1.3
Leucine	69.4^{B}	77.6^{A}	72.2^{AB}	75.4^{AB}	1.0
Lysine	50.6^{B}	67.1^{A}	67.2^{A}	62.2^{A}	1.8
Methionine	53.6^{b}	61.7^{a}	57.5^{ab}	53.1^{b}	2.0
Phenylalanine	71.1^{B}	80.6^{A}	69.2^{B}	77.6^{A}	1.1
Threonine	62.0^{b}	70.1^{a}	68.2^{ab}	68.6^{ab}	1.2
Valine	67.7^{b}	75.2^{a}	71.5^{ab}	69.2^{ab}	1.1
Dispensable					
Alanine	48.1^{B}	66.7^{A}	63.5^{A}	62.4^{A}	1.8
Aspartic acid	58.7^{B}	69.3^{A}	70.4^{A}	65.9^{A}	1.4
Cysteine	84.6^{a}	73.1^{b}	78.0^{ab}	84.2^{a}	1.3
Glutamic acid	81.9^{B}	86.1^{A}	80.6^{B}	85.5^{A}	0.7
Glycine	60.2^{B}	71.8^{A}	71.9^{A}	69.0^{A}	1.3
Proline	83.3^{B}	86.7^{A}	79.8^{C}	86.8^{A}	0.7
Serine	71.1^{b}	77.6^{a}	74.2^{ab}	76.3^{ab}	0.9
Tyrosine	60.7^{a}	67.0^{a}	64.6^{a}	66.7^{a}	1.2

[1]Standard error of the mean.

[2]Means within a given row not followed by a common superscript are significantly different:A,B,C at $P<0.01$; a,b at $P<0.05$

Table VI.7. True digestibilities (%) of crude protein and amino acids in the barley diets by pigs (exp. 2).

Barleys	Galt	Hiproly	Risø	Line 6	SEM[1]
Crude protein	72.5^{C}[2]	82.5^{A}	75.6^{BC}	80.1^{AB}	1.3
Amino acid					
<u>Indispensable</u>					
Arginine	84.9^{b}	89.0^{ab}	89.1^{ab}	90.4^{a}	0.7
Histidine	87.7^{b}	89.8^{ab}	89.9^{ab}	91.0^{a}	0.6
Isoleucine	74.0^{B}	84.9^{A}	77.1^{AB}	84.4^{A}	1.4
Leucine	78.0^{B}	86.1^{A}	80.9^{AB}	85.3^{A}	1.0
Lysine	62.9^{B}	78.4^{A}	75.9^{A}	76.3^{A}	1.7
Methionine	63.0^{b}	78.7^{a}	73.8^{a}	75.0^{a}	1.9
Phenylalanine	78.6^{B}	86.7^{A}	78.1^{B}	85.5^{A}	1.1
Threonine	75.5^{b}	83.2^{a}	79.0^{ab}	83.7^{a}	1.3
Valine	78.4^{a}	85.8^{a}	81.1^{a}	81.5^{a}	1.1
<u>Dispensable</u>					
Alanine	60.8^{B}	78.5^{A}	73.4^{A}	76.6^{A}	1.8
Aspartic acid	71.8^{b}	81.7^{a}	79.5^{a}	80.8^{a}	1.4
Cysteine	95.7^{B}	92.8^{BC}	89.3^{C}	100.3^{A}	1.0
Glutamic acid	86.1^{b}	90.1^{a}	86.3^{b}	90.1^{a}	0.6
Glycine	70.8^{B}	82.4^{A}	79.7^{A}	81.3^{A}	1.4
Proline	86.7^{AB}	89.8^{A}	85.1^{B}	90.4^{A}	0.6
Serine	80.3^{b}	86.7^{a}	82.7^{ab}	86.7^{a}	1.0
Tyrosine	71.7^{b}	78.1^{a}	75.4^{ab}	80.0^{a}	1.3

[1]Standard error of the mean.

[2]Means within a given row not followed by a common superscript are significantly different: A,B,C at $P<0.01$; a,b,c at $P<0.05$.

mutant Risø 1508 has expressed its genetic potential for improved AA content in different environments. The experimental barley, Line 6, is clearly a "high lysine barley" since its lysine content is positively correlated with its protein content (Munck et al. 1971; Doll et al. 1974).

Analyses of the barley protein (Table VI.1) indicated that only Risø 1508 could meet the total amino acid requirements for 60 to 100 kg pigs (NRC 1979). Chemical scores (CS) suggested that Hiproly and Line 6 were deficient in lysine, and methionine plus cysteine, i.e., for Hiproly, CS=92 and 88; for Line 6, CS=84 and 95, respectively; Galt was deficient only in lysine (CS=86). In practical pig diets, therefore, these barleys would require supplementation with varying levels of protein concentrates e.g., soybean and canola meals, to correct AA deficiencies.

Biological Evaluation

In the rat experiment, the RPV's, expressed as a percentage of that of Galt, were 109.3 for Hiproly, 108.2 for Risø 1508, and 115.8 for Line 6, i.e., an average of 11% improvement in the protein quality. These results agree very closely to the N balance data obtained in the pig experiment which showed a similar 11% average increase in the amount of N retained by pigs fed these same barleys.

The validity of the fecal analysis method (Kuiken and Lyman 1948) to estimate AA availability has been severely criticized in recent years because it does not consider the

influence of the microflora on the metabolism of protein residues in the hindgut. The use of this method for evaluating the availabilities of AA in high lysine barleys is based on previous pig experiments (Sauer and Just 1979, Sauer et al. 1979) which showed no significant differences between the AD of lysine and methionine, measured at the terminal ileum and over the total digestive tract.

The higher digestibilities (AD and TD) of crude protein of the barleys in rats as compared to pigs might be related to differences between these animal species. In contrast, Slump and van Beek (1977) found that the AD of N was higher in pigs than in rats when the same diets (corn- or wheat bran-based) were supplemented with different protein concentrates. Therefore, it would seem that the digestibility of crude protein was also related to the dietary composition. In the current study, the observation that AA digestibilities of the test barleys were similar to, or greater than those of Galt, would indicate that more AA were available to the pigs for absorption and subsequent protein synthesis when these test barley diets were fed. The amount (g) of available lysine (lysine intake,g x TD of lysine,%), was highest for pigs fed Risø 1508 (31.6), as compared to 22.9 for Hiproly, 20.4 for Line 6, or only 17.0 for Galt. These values are directly proportional to the observed biological values for the different barleys.

Helm (1977) reported the initial yield potential of Hiproly to be only 30 to 45% of the actual yield of a normal

control barley. If yields in the high lysine barleys could be improved to levels reported by Persson and Karlsson (1977), then these barleys could make a substantial contribution in reducing the amount of added protein supplements required in practical diets for monogastric animals. Jenkins et al. (1979) showed that yields in both normal and high lysine barleys, including Risø 1508, were significantly increased by agronomic practices such as irrigation and application of N fertilizers.

In conclusion, the Alberta grown high lysine barleys showed considerable promise. In chemical evaluation of the protein quality of high lysine barleys, it might be more appropriate to consider AA content, expressed as a percentage of the grain rather than as a percentage of the protein. This was exemplified by Line 6 which met the criteria of a high lysine barley and was superior to Galt, as measured by N balance and apparent biological value; however, the levels of AA, expressed as a percentage of the protein, would suggest similar protein quality in both of these barleys. Consequently, it is imperative that biological evaluation be conducted with animals, particularly with those livestock species in whose diets the barleys would be included.

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VII. GENERAL DISCUSSION

A. NITROGEN METABOLISM IN THE HINDGUT OF THE GROWING PIG

Prior to the use of intestinal cannulation as a technique in nutritional metabolic studies, it was thought that residual dietary constituents leaving the end of the small intestine passed through the hindgut largely unchanged. Analyses of ileal digesta and feces from chickens (Payne et al. 1968), rats (Varnish and Carpenter 1971), pigs without cannulas (Cho and Bayley 1972, Mason et al. 1976) and pigs fitted with cannulas (Zebrowska 1975, Sauer 1976, Tanksley and Knabe 1980) provided clear evidence that the hindgut was the region of intense microbial activity, involving the metabolism of carbohydrates and protein residues. Gargallo and Zimmerman (1981) estimated that the hindgut of the pig could digest up to 150 g starch/d. These and other workers (Just et al. 1981) showed that the hindgut possessed a high capacity for the digestion of nitrogenous substrates. Other workers (e.g., Mason et al. 1976, 1977) showed that N metabolism in the hindgut was energy-dependent. In the current study, it was demonstrated that the extent of microbial activity, as measured by output of fecal N, was affected by the presence of fermentable energy substrates (cornstarch, pectin and wheat bran), and dietary protein quality (meat-and-bone meal vs soybean meal). When energy was limiting for the microbes (water infusion), protein residues in the hindgut were degraded to

constituent carbon skeletons and ammonia (Hodgdon 1977). The latter was absorbed into the hepatic portal blood (Hodgdon 1977) and excreted largely as urea in the urine. These effects were more pronounced when the dietary protein was of lower digestibility, as exemplified in the present study by meat-and-bone meal as compared to soybean meal. Infusion of carbohydrates which provided energy in excess of the digestive capacity of the hindgut (Gargallo and Zimmerman 1981) elicited a quantitative change in the pathway of N excretion. In this case, the ammonia produced from protein degradation (Hodgdon 1977) was utilized for de novo synthesis of bacterial protein which was voided in the feces. As a consequence, less ammonia was absorbed into the blood for conversion into urea and excretion via the urine. Since the conversion of ammonia to urea requires the expenditure of energy (Lehninger 1975), the presence of energy substrates in the hindgut may result in a net saving of metabolic energy to the animal. The observation of no decrease in the urinary urea output when pigs were fed the highly digestible cornstarch-soyprotein diet and infused with cornstarch would suggest that N might still be limiting for hindgut microbes, despite the recycling of endogenous N (Holmes et al. 1974, Mosenthin 1981).

Infusion of protein as soyprotein and wheat bran substantiated previous reports of the high capacity of the hindgut for protein digestion (Just et al. 1981, Gargallo and Zimmerman 1981); however, the value of the N end

product(s) was of little significance to the nutrition of the pig since neither N retention nor apparent biological value was improved.

The wide variation in the apparent digestibilities of the individual amino acids from that of the respective crude protein (Chap. II and III) indicated beyond doubt that the digestibilities of the amino acids, and not crude protein, were more meaningful, and therefore must be considered when formulating practical diets.

For years, protein from different feed ingredients (of plant or animal origin) were thought to have the same nutritional value. Practical diets for domestic livestock have been prepared on the basis of crude protein content to meet the requirements for amino acids. Subsequently, this procedure was refined and improved by using dietary amino acid content; however, the amount of individual amino acids actually available for protein synthesis is often ignored. Results of this study indicated that the fecal analysis method (Kuiken and Lyman 1948) did not give reliable measures of amino acid availabilities because it failed to take into account the metabolism of N in the hindgut. When energy was limiting (water infusion), amino acid availabilities were overestimated, whereas when energy was adequate or in excess, amino acid availabilities were underestimated. If amino acids are provided in diets formulated using such values, then in the former case, the pig would be supplied with lower than adequate levels of

amino acids for optimal growth; in the latter case, reduced feed conversion efficiency (and therefore feed wastage) would result. It is obvious, therefore, that ileal digestibilities are more valid estimates than those values determined over the total digestive tract (Sauer 1976, Zebrowska 1978, Tanksley and Knabe 1980).

B. COMPOSITION AND UTILIZATION OF HIGH LYSINE BARLEYS BY GROWING RATS AND PIGS

Although commonly classified as energy sources, cereal grains are also the world's major protein sources; however, cereal proteins are low in the level the indispensable amino acid lysine (Munck et al. 1971, Sauer 1976, Eggum 1977) which limits their nutritional value to monogastric animals. Concerted world wide efforts to change the amino acid composition of cereal proteins by genetic manipulation in order to meet more adequately the amino acid requirements of monogastric animals including man, represent the culmination of at least 50 years of research (Munck 1976). Significant contributions in the development of high lysine barleys and their evaluation (chemical and biological) have been made by scientists working in India (Balaravi et al. 1976, Bansal et al. 1977), Scandinavia (Munck et al. 1970, 1971, 1975; Ingversen et al. 1973, Ingversen and Koie, 1973, Koie et al. 1976, Olsen 1980, Olsen and Krekling 1980), the United Kingdom (Johnson et al. 1978, Jenkins et al. 1979, Rhodes and Gill 1980), the United States of America (Alexander et

al. 1979, Newman et al. 1979) and Canada (Beames 1977, Misir and Sauer 1981c). In Alberta the barley breeding program at the Lacombe Agricultural Research Station is aimed at the development and propagation of nutritionally superior high lysine barley lines adapted to local soil and climatic conditions, utilizing existing high lysine barleys, especially Hiproly (Munck et al. 1971) and Risø 1508 (Ingversen et al. 1973). Chemical and biological evaluations are conducted at the Department of Animal Science, University of Alberta (as discussed in this dissertation).

The results of the present study showed that locally developed high lysine barley lines, notably Line 1 (Chap. V) and Line 6 (Chapter VI) compared favorably with Hiproly and Risø 1508, in terms of both chemical composition and biological evaluation. Greater amounts (g) of lysine were available to the pigs fed the high lysine barleys than the control, Galt (Chapter VI). It is noteworthy, too, that the protein quality of Hiproly, Risø 1508 and Line 6 were found superior to Galt in experiments with both rats and pigs, thus indicating that feeding trials with rats could be useful as preliminary studies on nutritional quality when the supply of test berleys is limited.

It must be emphasized that the increase in the lysine content was achieved by an alteration of the proportions of the different protein fractions (Ingversen et al. 1973, Balaravi et al. 1976, Munck 1976). Increased lysine content was also accompanied by increases in the levels of threonine

(Ingversen et al. 1973, Beames 1977, Helm 1977). If as suspected, this improvement is achieved without a lowering of the concentration of the energy components in the grain, then the use of high lysine barleys in practical diets for domestic monogastric species will certainly increase efficiency in livestock production. Perhaps, the only major problem to overcome is the low yields, as reported by some workers (Munck et al. 1970, Ingversen et al. 1973, Helm 1977); however, others, e.g., Persson and Karlsson 1977, have found yields comparable to high yielding control barleys. Furthermore, yields could be increased by good agronomic practices, such as irrigation and application of N fertilizers (Jenkins et al. 1979). The current study has shown that high lysine barleys, bred in Scandinavia also express the high-lysine trait in Alberta. Therefore, the prospects for large scale production and utilization of high lysine barleys in diet formulation certainly look promising. To achieve these objectives suitable high lysine barley seeds must be made available to farmers who should then be paid a premium for the amount of such barleys marketed.

In Alberta, the barley production amounted to 5.9×10^6 tonnes, i.e., 45% of the total cereal grain harvested (Alberta Agriculture 1980). Since barley is the grain most commonly used in pig diets, the use of high lysine barleys will reduce the need for added protein ingredients (soybean meal, canola meal) which can then be diverted to other uses e.g., human nutrition. Both the farmers and livestock

producers may be direct beneficiaries of high lysine barley research. At the present time, the use of existing high lysine barleys as sole protein, and possibly energy source, may be justified only for finishing pigs (60 to 100 kg). Diets for growing pigs would require supplementation with a protein concentrate, though at a reduced level than when normal barleys are fed.

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VIII. GENERAL CONCLUSIONS

The objectives of this thesis were to evaluate:

(A) The role of the hindgut of the growing pig in N metabolism in order to reevaluate amino acid digestibility measurements (fecal analysis) as valid estimates of amino acid availabilities, and

(B) The protein nutritional quality of Alberta grown high lysine barleys by chemical and biological methods.

A. ROLE OF THE HINDGUT OF THE GROWING PIG IN NITROGEN METABOLISM

1. Microbial activity was dependent on the amount of energy substrates and protein residues in the hindgut. In practical feeding regimes, this would depend on the respective digestibilities of these ingredients, measured at the terminal ileum.
2. Enhanced microbial activity was accompanied by a change in the pathway of N excretion. Fecal N was increased and total urinary N was decreased. Fecal and urinary losses are thus not completely discrete entities. The predominant N end product of protein digestion, most probably ammonia, was absorbed into the blood and excreted largely as urea in the urine.
3. The hindgut has a high capacity for protein digestion; however, the N end products did not contribute to the N status of the pigs fed diets adequate in protein.
4. Wide variation in the apparent fecal digestibilities of amino acids from that of the dietary protein clearly

indicated that crude protein digestibility was of little or no value in formulating diets to meet the pig's requirements for amino acids.

5. The fecal analysis method overestimated or underestimated amino acid availabilities; therefore, it would be more appropriate to measure amino acid availabilities from digesta taken at the terminal ileum.
6. In practical cereal based diets, the associative effects of undigested carbohydrate and protein residues entering the hindgut must be considered in order to accurately measure amino acid availabilities (fecal analysis method).

B. EVALUATION OF HIGH LYSINE BARLEYS

1. Chemical analyses showed that Alberta bred high lysine barley lines, an established high protein high lysine barley (Hiproly), and a high lysine barley mutant (Risø 1508), all grown in Alberta, are superior to a normal control barley (Galt).
2. Feeding experiments with both rats and pigs attest to the superior nutritional quality of their protein.
3. In practical pig diets, some of these barleys, e.g., Line 1 and Risø 1508, may meet the total amino acid requirements for 60 to 100 kg pigs; others, e.g., Hiproly and Line 6, would require supplementation with protein ingredients, but at reduced levels, as compared to normal barleys.